The Dissertation Committee for Jung Su Park Certifies that this is the approved version of the following dissertation:

Biodegradation of Paint VOC Mixtures in Biofilters

Committee:

Kerry A. Kinney, Supervisor

Gerald E. Speitel, Jr

Richard L. Corsi

Christian E. Whitman

Frank A. Seibert

Biodegradation of Paint VOC Mixtures in Biofilters

by

Jung Su Park, B.S., M.S.

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

December, 2004

Dedication

To my wife Soojin

and my parent.

Thank so much for all you love and support.

Acknowledgements

I would like to express my sincere gratitude to my advisor Dr. Kerry A. Kinney for her advice and support throughout my master and doctoral works. She always made time to listen and provide prudent advice when I needed advice and encouragement. I also would like to thank to my committee members, Dr. Gerald E. Speitel Jr., Dr Richard L. Corsi, Dr. Christian P. Whitman and Dr. Frank A. Seibert for providing me guidance along the way.

I also want to thank past and current members of Dr. Kinney's research group for all of his or her help in the laboratory. Song, Jennifer, Susan, Soondong, Federico, Kate and Lily. I would like to recognize Claudia and Katy for their help in my molecular experiments. I also thank to SRP (Separation Research Program) people for their technical advice and physical helps on setting up the pilot scale column.

I would like to thank my parents and all my family for supporting me and encouraging me. Last but not least, I thank my wife for her love and for always believing in me.

Biodegradation of Paint VOC Mixtures in Biofilters

Publication No._____

Jung Su Park, Ph.D.

The University of Texas at Austin, 2004

Supervisor: Kerry A. Kinney

The surface coating industry is facing increasingly stringent environmental regulations that require the control of volatile organic compounds (VOCs) and hazardous air pollutants. Biofiltration is an attractive alternative for paint spray booth applications but these facilities pose several challenges to biofiltration technology . Specifically, off-gas streams from painting operations are characterized by complex VOC mixtures, frequent shutdown and restart events, high volumetric flow rates, and relatively low contaminant concentrations. The objectives of this research were to investigate the feasibility of biofiltration for paint spray booth applications and to delineate how key operating parameters and biofilter history affect the degradation of VOC mixtures and the microbial population in biofilters. The results indicate that biofilters are a feasible option for treating the emissions from paint spray booth facilities. Removal efficiencies as high as 95% or greater were achieved even under intermittent feed conditions. Biofilter performance was found to strongly depend on nitrogen supply, particularly during the start up period. Overall VOC removal was limited by the toluene and xylene components of the waste gas stream. When the inoculating culture was developed so as to maintain

the degradation capacity of the culture for each VOC component of the paint mixture, a sequential feeding strategy did not provide any initial advantage with respect to VOC removal; however, the system ultimately achieved higher removals of the toluene. Biofilm samples analyzed using the DGGE technique indicate that the fungal population in the bioreactors was relatively uniform across the biofilters and stable over extended periods of operation. The DGGE banding pattern for the bacterial population, on the other hand, indicates that the bacterial community was spatially distinct and became less diverse after 200 days of operation. While this research focused on biofilters treating paint VOC mixtures, it is anticipated that many of the phenomena observed will be applicable to other bioreactor systems and contaminant mixtures.

Table of Contents

List of Tables

List of Figures

Chapter 1 Introduction

Regulatory standards for the emissions of hazardous air pollutants from various sources have intensified in response to the Clean Air Act Amendments of 1990. To meet these stringent regulations, various off-gas treatment methods such as incineration and absorption have been applied. These physicochemical processes are often most effective for moderate off-gas flow rates and high contaminant concentrations (greater than 5 g/m^3) but become less cost-effective when treating relatively dilute contaminant streams.

Biological methods for treating off-gas streams using vapor-phase bioreactors (VPBs) have emerged as a viable treatment technology for the emissions of volatile organic compounds (VOCs). Vapor phase bioreactors are well suited to treat dilute contaminant streams and numerous studies have demonstrated that VPBs can effectively degrade a wide variety of VOCs ranging from aliphatic compounds to monocyclic aromatics and chlorinated species. These VPBs are attractive for several reasons, including minimal waste production and low operating costs particularly as compared to conventional, physicochemical treatment methods. These techniques are gaining industrial confidence, and systems are now under development to expand their application range to higher concentrations and gas flow rates.

1.1 BACKGROUND

The surface coating industry is facing increasingly stringent environmental regulations that require the control of volatile organic compounds (VOCs) and hazardous air pollutants (HAPs). Paint spray booths, in particular, emit large quantities of VOCs such as toluene, xylene and methyl ethyl ketone. Several of these components are

classified as HAPs in the 1990 Clean Air Act Amendments (CAAA) because they are known or suspected to cause adverse human health affects. Even VOCs that are not among the 188 HAPs listed in the CAAA can have human health impacts. For instance, many VOCs react photochemically with nitrogen oxides in the atmosphere to form tropospheric (low-level) ozone which contributes to respiratory dysfunction in humans.

Although conventional VOC control technologies such as thermal incineration and adsorption can be effective at reducing emissions from surface coating operations, they generate undesirable byproducts, are energy intensive and may not be cost-effective when treating high flow air streams contaminated with low concentrations of pollutants. Biofiltration is an attractive alternative for low concentration gas streams because of its low energy consumption, relatively moderate operating costs and minimal byproduct generation. In this technology, contaminants from the air stream are transferred into a biofilm immobilized on the packing support media and converted by the microorganisms into products such as carbon dioxide, water and additional biomass. Biofiltration technology has been used to treat relatively low concentrations of odorous compounds and volatile chemicals from off-gas streams in wastewater treatment plants, composting operations, rendering plants, and chemical manufacturing facilities. To date, however, there have been limited studies of biofiltration for the treatment of off-gas streams from paint booth operations. Although biofiltration is an environmentally friendly technology that holds great promise for this application, several challenges need to be overcome. Specifically, off-gas streams from painting operations are characterized by complex VOC mixtures, frequent shutdown and restart events, high volumetric flow rates, and relatively low contaminant concentrations. Thus, it is necessary to develop and optimize an innovative biofilter system specifically for this application so that reliable and efficient bioreactor operation can be achieved.

1.2 PROBLEM IDENTIFICATION

To date, most biofiltration studies have focused on the treatment of a single pollutant to minimize the complexity of the system and to elucidate the effect of basic operating parameters on performance. However, when more than one compound is present in a biofilter system, the response of the system is more complicated due to possible microbial and substrate interactions. Common examples of chemical mixtures include gasoline and other petroleum fuels, pesticides, wood-treating substances, and paint solvents. Although relatively extensive studies of BTEX biodegradation have been conducted, few studies have been completed to investigate paint spray mixtures even though paint spray booths are a major VOC emission source. Since organic wastes from industrial and municipal sources are often present as mixtures, the response of biofilters to these mixtures must be investigated. In particular, the effect that nutrient availability and VOC acclimation history have on the microbial community and, ultimately the degradation capacity of bioreactors treating VOC mixtures should be addressed.

The practical application of biofiltration to paint spray booths faces other challenges in addition to complicated substrate and microbial interactions. Paint booths operate intermittently, which makes it more difficult to apply biological treatment techniques. Also, paint spray booths generate gas streams that are characterized by dynamically varying VOC concentrations and gas flow rates from these systems can range from a few thousand scfm to 100,000 scfm or greater. These properties of paint spay booths increase the difficulty of treating paint emissions in biofilters.

1.3 RESEARCH OBJECTIVES

The main objectives of this research were to investigate the feasibility of biofiltration for paint spray booth applications and to delineate how key operating parameters and biofilter history affect the degradation of VOC mixtures and the microbial population in biofilters. Specific objectives included the following:

- Investigate the degradation pattern of paint VOC mixtures and develop strategies to minimize any negative substrate inhibitions observed;
- Characterize the spatial and temporal variation in the microbial population within a biofilter as a function of nitrogen availability and acclimation history and investigate how any microbial population shifts correlate to changes in VOC degradation patterns; and
- Examine the feasibility of biofiltration for paint spray booth applications under more realistic conditions including complex paint mixtures and intermittent operation.

1.4 RESEARCH APPROACH

This research investigated the feasibility of biofiltration for paint spray booth applications. Biodegradation of multiple organic compounds is more complicated due to possible substrate interactions and microbial competition. Since paint spray booths generate a complex mixture of readily degradable and relatively recalcitrant compounds, it is important to investigate the degradation pattern of each compound in the mixture and identify the factors which control overall VOC removal in biofilters. The research was divided into the following five individual tasks

- Task 1: Conduct baseline biofilter experiments to identify key parameters affecting removal of paint VOC mixtures. (Chapter 3)

- Task 2: Examine the effect of acclimation method on the degradation of paint VOCs and the composition of the microbial population in the biofilter. (Chapters 4 and 5)
- Task 3: Investigate the impact of nitrogen supply on the degradation of paint VOC mixtures by monitoring substrate degradation patterns and microbial diversity in the biofilm. (Chapter 6)
- Task 4: Evaluate biofilter performance under transient feed conditions and over a range of operating conditions such as empty bed contact time and inlet VOC concentration (Chapter 7)
- Task 5: Examine the effect of complex VOC mixtures on biofilter performance in a pilot scale bioreactor treating actual paint emissions. (Chapter 8)

At the beginning of this research, a series of baseline biofilter experiments were conducted to identify key operating parameters affecting the removal of paint VOC mixtures. Based on the findings of these baseline experiments, a significant portion of the subsequent research focused on better understanding how VOC acclimation history and nitrogen availability affect VOC degradation and the microbial community in the biofilters. It was hypothesized that the order that VOCs are provided to a biofilter during startup may affect the ultimate VOC removal capacity achievable in the system as well as the composition of the microbial population within the bioreactor. Most biofiltration studies to date have neglected to investigate in a systematic way how the operational history of a biofilter affects subsequent performance of these biological systems. Particularly after long periods of operation, the microbial community present in a biofilter may be very different than the one present at the beginning of operation when most biofilter experiments are conducted. Thus, a series of experiments were

conducted in this research to determine the effect of inoculum development, substrate feeding history, and nitrogen conditions on VOC degradation patterns and the diversity and stability of the microbial population in biofilters treating paint VOC mixtures

Finally, the response of the biofilter was examined under more realistic and challenging conditions such as treating intermittent waste streams containing complex mixtures of pollutants. A series of lab-scale studies were conducted to determine the response of a mature biofilter to transient feed conditions and to delineate the operating range of biofilters for paint applications with respect to gas phase residence time and VOC concentration. These laboratory-scale studies laid the foundation for developing a pilot-scale biofilter which was investigated for its ability to treat actual paint emissions on an intermittent basis. Scale-up issues such as bioreactor start-up procedures as well as slip feed design were also investigated in the pilot-scale studies. Successful completion of this research provides a more fundamental understanding of multiple VOC degradation in biofilters and also elucidates the effects of biofilter history and operating parameters on the performance of biofilters treating VOC mixtures.

Chapter 2 Background and Literature Review

Biofiltration is a promising air pollution control technology in which microorganisms growing on a fixed packing material are used to degrade volatile organic compounds and remove odors. Biofiltration of single pollutants such as toluene has been studied extensively to elucidate basic phenomena in vapor phase bioreactors, and this process is considered a cost-effective alternative to conventional air pollution control methods. However, when more than one compound is present in a biofilter system, both substrate and microbial interactions as well as operating parameters such as packing media, nutrients, pH and moisture content play an important role in the biodegradation of multiple substrates.

Section 2.1 provides a general overview of biofiltration technology including a description of the common types of vapor phase bioreactors and their practical application. Sections 2.2 delineates the methods used to assess bioreactor performance and Section 2.3 identifies key bioreactor operating parameters that affect this performance In Section 2.4, multiple substrate degradation and microbial population studies are reviewed since microbial competition is expected to occur in biofilters treating VOC mixtures such as those found in paint booth emissions. Section 2.5 presents microbial monitoring techniques with a focus on the DGGE (Denaturing Gradient Gel Electrophoresis) monitoring method.

2.1 Overview of Biofiltration

The biofiltration process can be divided into three basic steps. First, a pollutant in the gas phase is passed through a biologically-active packed bed. The pollutant then diffuses into a biofilm immobilized on the packed medium. Finally, microorganisms

growing in the biofilm oxidize the pollutant as a primary substrate or co-metabolite and in the process convert the contaminants into the benign end products of carbon dioxide, water and additional biomass (Swanson and Loehr 1997; Madigan 2000).

This technology is attractive for many reasons including its ability to convert pollutants to inert products such as CO_2 and H_2O at ambient temperatures. Another advantage of biofilters is that they do not generate secondary contaminant problems and thus are an environmentally friendly treatment method. Biofilters and biotrickling filter can be a more cost-effective option than conventional air pollutions control methods for high volume, low concentration gas streams containing readily biodegradable contaminants (van Lith *et al*., 1997). Finally, because these systems operate at ambient temperatures and do not require high-temperature media regeneration systems, they have lower energy requirements than competing technologies.

2.1.1 Basic Types of Vapor Phase Bioreactors

Three primary bioreactor configurations are available to treat stationary sources of air pollution. These various reactor configurations are generally referred to as biofilters, bioscrubbers, and biotrickling filters (Ottengraf, 1987; van Groenestijn and Hesselink, 1995). Each technology operates under different conditions as summarized briefly in Table 2-1.

Table 2-1: Classification of bioreactors for waste gas treatment (Ottengraf, 1987).

Reactor Type	**Microorganisms**	**Aqueous Phase**
Biofilter	Fixed	Stationary
Biotrickling Filter	Fixed	Flowing
Bioscrubber	Suspended	Flowing

Biofilters (Figure 2-1) are the oldest and simplest method of the three vapor-phase bioreactors and involve passing a contaminated air stream through a reactor containing biologically-active packing material. The contaminants are transferred from the air stream into a biofilm immobilized on the support media and are converted by the microorganisms into carbon dioxide, water, and additional biomass. Moisture is typically supplied to the biofilm in a humid inlet waste gas stream. Packing media used in biofilter beds can be broadly categorized as either "natural" or "synthetic". Natural media include wood chips, peat, and compost with compost being the most widely used. Synthetic media include activated carbon, ceramic pellets, polystyrene beads, ground tires, plastic media, and polyurethane foam (Moe and Irvine, 2000, 2001). Natural organic packing media generally contains a supply of nutrients (i.e., nitrogen, phosphorus, and other elements necessary for microbial growth) as a naturally occurring component of the packing itself. When a synthetic support medium is used, nutrients must be added for microbial growth. Nutrients may be mixed with the packing material before biofilter assembly or supplied via a solution sprayed on or mixed with the packing material after construction. Even if a nutrient solution is periodically sprayed onto the packing material, a continuous flowing liquid stream is not present in biofilter systems (Devinny *et al.*, 1999; van Groenestijin and Hesselink, 1994).

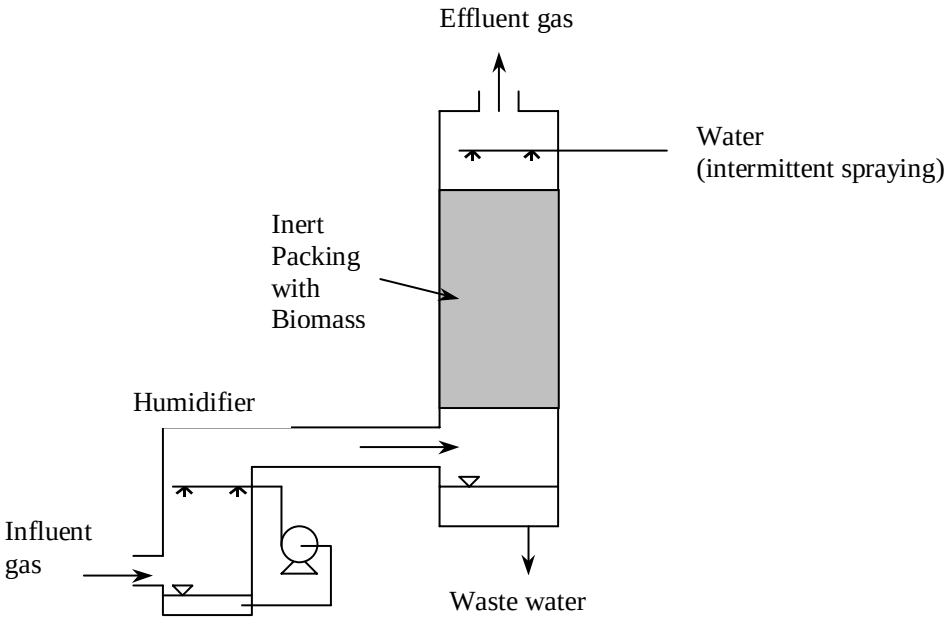


Figure 2-1: Schematic diagram of a biofilter (Van Groenestijn and Hesselink 1994).

Biotrickling filters (Figure 2-2) are similar to biofilters with the exception that there is a liquid nutrient medium continuously recirculating through the column. To facilitate recirculation of the liquid phase, rigid synthetic media (e.g., plastic media, polyurethane foam, or lava rock) is used as the packing medium. Microorganisms grow primarily as a fixed film on an inert packing media; however, microorganisms are also present in the liquid phase both because they can grow suspended in the liquid and because the flowing liquid imparts sufficient shear force to detach biomass from the solid support media. The air and liquid streams can move either co-currently or counter-currently depending on the operating conditions. Contaminants are transferred from the air stream into the liquid phase and biofilm for subsequent degradation (Devinny *et al*., 1999; van Groenestijin and Hesselink, 1994). As compared to conventional biofilters,

biotrickling filters offer the advantages of increased operator control over key parameters such a nutrient concentrations and pH, as well as the opportunity to wash degradation by-products out of the reactor (Devinny *et al.*, 1999; van Groenestijin and Hesselink, 1994). A potential disadvantage of biotrickling filter operation, however, is that clogging of the pore space can occur if the biotrickling filter is treating high VOC loads and is provided excess nutrients (Webster *et al.*, 1998a; 1998b; Sabo *et al.*, 1998, Cox and Deshusses, 1997; Sorial *et al.*, 1995). An additional disadvantage to biotrickling filter operation as compared to "classic" biofilters is the need to manage the liquid stream. Furthermore, the specific surface area in biotrickling filters is generally lower than in biofilters (Ottengraf, 1987); therefore, biotrickling filters may have more difficulty treating poorly soluble compounds.

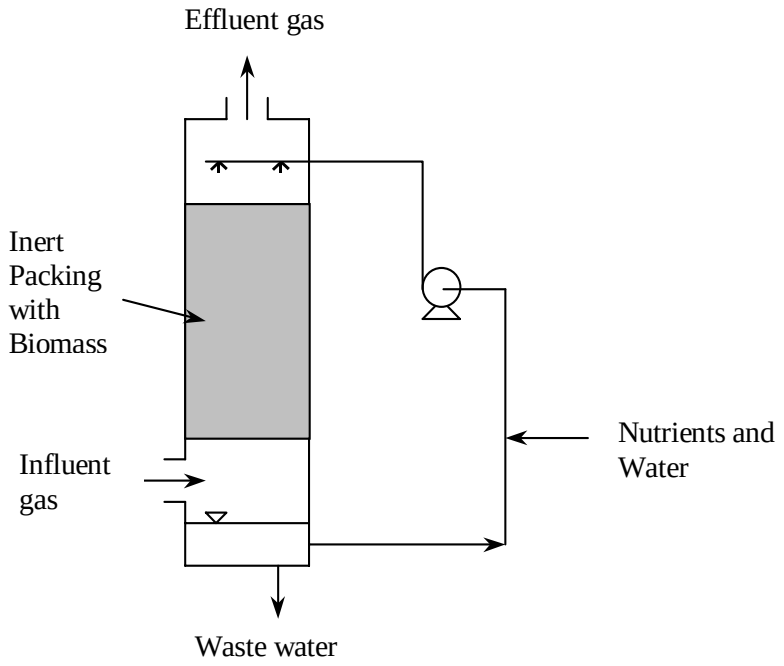


Figure 2-2: Schematic diagram of a biotrickling filter (Van Groenestijn and Hesselink 1994).

Bioscrubbers (Figure 2-3) combine physical-chemical treatment with biological treatment using two separate reactors to accomplish treatment. In the first reactor, the contaminated air stream is contacted with water in a reactor packed with inert media, resulting in contaminant transfer from the air phase to the liquid phase. The liquid is then directed into an activated sludge reactor where the contaminants are biologically degraded (van Groenestijin and Hesselink, 1994; Ottengraf, 1987). The separate activated sludge tank allows the reactor to treat higher concentrations of compounds than biofilters can handle. In addition, since compound transfer and degradation occur in separate reactors, optimization of each reactor can take place separately. As with

biotrickling filters, bioscrubbers offer greater operator control over nutrient supply, acidity, and the build up toxic by-products. A potential disadvantage of bioscrubbers over biofilters, however, is that slower growing microorganisms may be washed out of the system and disposal of excess sludge is required. (Kok, 1992)

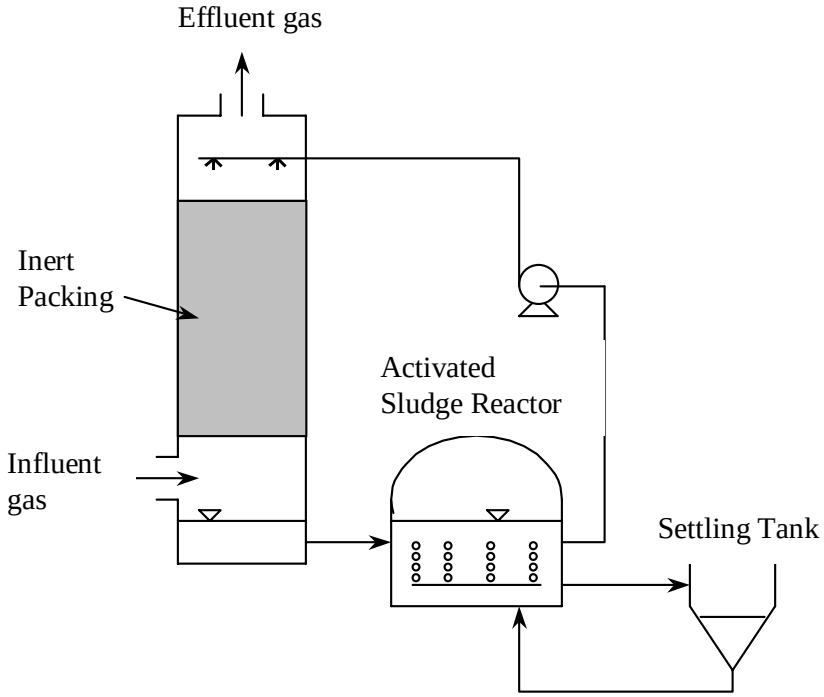


Figure 2-3: Schematic diagram of a bioscrubber (van Groenestijn and Hesselink 1994).

2.1.2 Mechanisms of VOC removal

The biodegradation of pollutants in the biofilm of a biofilter or biotrickling filter consists of two steps: (1) mass transfer of the target pollutant from the gas phase to the liquid biofilm phase containing microorganisms, and (2) biological degradation of the

pollutants as carbon and energy sources for the microorganisms within the biofilm. Figure 2-4 illustrates the mass transfer and biodegradation processes occurring at the microscale level.

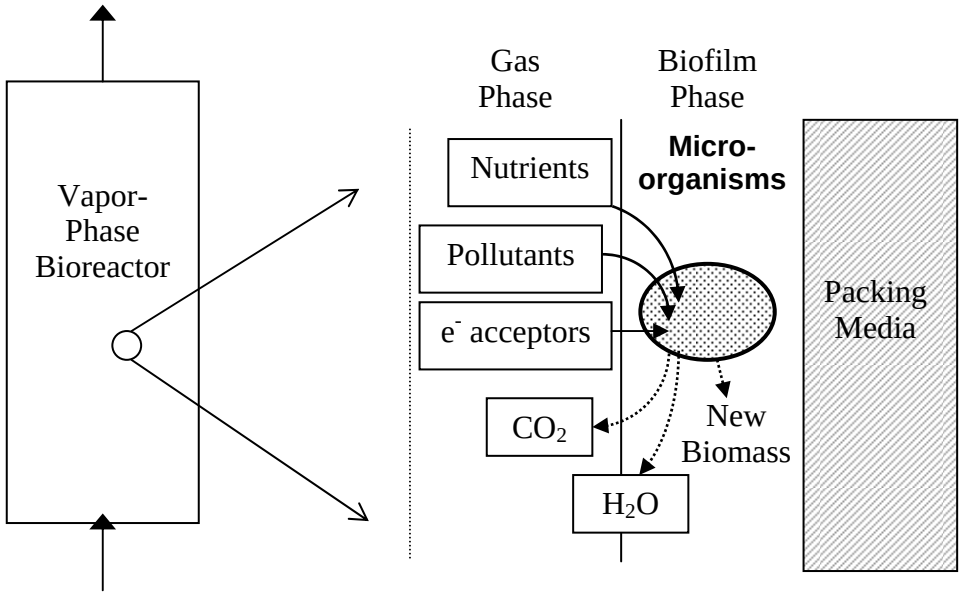


Figure 2-4: Biodegradation processes and biological reactions in a VPB.

In order for biodegradation to occur in a biofiltration system, the pollutant must be transferred to the biofilm phase. Less soluble substances need a large gas/liquid surface area to obtain sufficient pollutant mass transfer for efficient destruction in vapor phase bioreactors.

2.1.3 Biofilter Applications

Historically, biofiltration has been most commonly applied to remove odorous compounds such as H_2S from air emissions at wastewater treatment plants. Since the 1980's, however, biofiltration has also been used to eliminate VOCs in gases from a wide

range of processes (van Groenestijn and Hesselink, 1995; Leson and Winer, 1991). A summary of the industries that have used biofiltration techniques can be found in Table 2-2.

Table 2-2: Industries using biofiltration.

Chemical Operations	Coffee Roasting
Composting Facilities	Chemical Storage
Coca Roasting	Landfill Gas Extraction
Film Coating	Fish Frying
Slaughter Houses	Investment Foundries
Flavors and Fragrances	Tobacco Processing
Print Shops	Pet Food Manufacturing
Waste Oil Recycling	Industrial and Municipal Wastewater Treatment Plants

(Lesson, 1991; Friedrich *et al*., 2003)

All of these sources typically emit large volumes of off-gases that contain only low concentrations of the target organic compounds. Table 2.3 presents an abbreviated list of chemicals that can be treated by biofiltration.

Table 2-3: List of chemicals treatable by biofiltration

Acetate	Dimethyl sulfide	Methyl mercaptan
Acetone	Ethanol	Nitrogen oxide
Ammonia	Ethylbenzene	Nitrogen dioxide
Benzene	2-Ethyl hexanol	Pentane
Butanol	Hexane	Scatole
Butylaldehyde	Hydrogen sulfide	Styrene
Butyl Acetate	Indole	Tetrachloroethene
Carbon monoxide	Iso-propanol	Thiophene
Mono-, Di-, Tri-chloromethane	Methane	Toluene
Diethyl amine	Methanol	Trichloroethene
Dimethyl disulfide	Methyl-ethyl-ketone	Xylene

(Lu *et al*., 2002; Li and Moe, 2003; Demeestere *et al*., 2002; Fitch *et al*., 2002; Apel *et al*., 1995; Barshter *et al*., 1993; Ergas *et al*., 1995; Hodge *et al*., 1991; Morgenroth *et al*., 1995; Mueller, 1998; Ottengraf and VanDenOever, 1983)

2.1.4 Transient Loading Conditions

Vapor phase bioreactors have proven to be an effective technology for removing odors and volatile chemicals. However, reliable long-term performance can be elusive in these systems, particularly when they are subjected to dynamic feed conditions or shutdown and restart conditions. In particular, loss of biomass activity during shutdown periods or inactive zones within the bioreactor itself can lead to poor performance. This transient loading situation is very common in paint spray booth operations. Reliable biofilter performance during transient chemical loading needs to be achieved if biofilters are to be applied to paint spray booths.

Several studies have shown that a substantial reduction in biomass activity occurs under carbon-deprived conditions (Martin 1996). In a study involving a bioscrubber, weekend intermissions resulted in a 30 to 60% decrease in biomass activity even though the contaminant elimination efficiency was restored again after two days (van Groenestijin 1994). Martin (1996) demonstrated that a biofilter required 9 to 24 hours to recover full biomass activity after a four-day shutdown (Martin 1996). Wani (1998) also investigated the re-acclimation time required after a period of non-use in a biofilter degrading methyl mercaptan, a malodorous gas produced from kraft pulping processes. Even though the 1-day re-acclimation period observed was much shorter than the initial start-up period of 5 to 6 days, the initial removal efficiency was so low that a backup treatment system would be needed to meet stringent regulations (Wani 1998).

In addition to the effect of shutdown periods on microbial systems, microbial activity can easily be affected by non-steady operating conditions. Lab-scale experiments reveal that when operating conditions are changed, more than three days are required to

reach a new steady state (Tang 1996). In another study, biological activity was found to be suboptimal under unsteady state environmental conditions even though biotrickling filters are not affected by a few hours of down time (Choi 1998).

Even though filter beds can survive starvation periods of at least two weeks without any permanent reduction in microbial activity, periodic aeration of the filter or operation of the blower in a turndown mode is advisable during shutdown periods to avoid oxygen starvation and/or dehydration in the filter (Ottengraf 1986). A slip feed system can also be introduced to reduce the re-acclimation time following shutdown period in a biofilter. A slip feed system which supplied a low-flow, surrogate slip gas stream to the bioreactor over a 3-day and a 7-day shutdown period was found to reduce the re-acclimation time required following restart by as much as 70 % (Park 2001). Generally, the longer the period of non-use, the greater the re-acclimation period required before the bioreactor removal efficiency was fully restored. Such periodic bioreactor shutdowns are common in field situations and slow recovery of bioreactor systems is a major drawback hindering their widespread application.

2.1.5 Paint Spray Booth Applications

Equipment maintenance operations are one of the greatest sources of VOC emissions at U.S Air Force installations and paint spray booths are considered the largest emissions source within this category (McMinn 1992; Whitfield 1993). According to National Emission Trends (NET) database from the U.S. EPA, total estimates of annual emissions of VOCs into the air from stationary and mobile sources in the U.S. were approximately two million tons nationally in 1999. Annual emissions of VOCs from coating and allied facilities were estimated to be 26,500 tons. Commonly used organic solvents include aromatics, acetates, ethers, and ketones, and nearly one hundred types of

solvents are used (Hsu 2000). Conventional VOC control processes such as wet scrubbing and activated carbon are limited for this application due to the low water solubility of some of the solvents and the high replacement cost of activated carbon.

Kim *et al*. (2000) investigated the feasibility of using a biological treatment process for gaseous VOCs emitted from automotive painting operations. Comparing the biological VOC removal process with vapor-phase adsorption/thermal oxidation, the biological process was found to be an order of magnitude more cost-effective in capital costs and a factor of two more cost-effective in O&M costs. Biological processes achieved similar overall VOC removals comparing to adsorption/thermal oxidation (33 to 36 % VOC removal by the biological process and 27 to 42 % VOC removal by the carbon adsorption/thermal oxidation). Even though all the hydrophilic solvents were captured in a bench-scale activated sludge reactor and biologically degraded, the hydrophobic compound removal was very poor (Kim 2000).

A few other studies have been conducted to investigate biofilters and biotrickling filters treating paint VOCs (Webster 1998; Hsu 2000; Kazenski 2000; Boswell 2001). Boswell *et al*. (2001) described a full-scale biofilter used to treat the off-gas stream from a paint production unit at a paint manufacturing facility. The VOC mixture was dominated by toluene, xylene, methyl ethyl ketone (MEK), acetone and ethylbenzene. They achieved approximately 60% overall VOC removal in the biofilter and demonstrated that the capital cost of the biofilter was significantly less than that of a thermal oxidizer. Also, operating costs were less than 10% of a comparably sized regenerative oxidizer (Boswell 2001). Hsu *et al.* (2000) demonstrated that a pilot-scale biofilter could achieve up to 95% overall VOC removal when the major organic compounds were xylene, toluene, methyl ethyl ketone (MEK), iso-propanol, and iso-butanol. Annual construction and operation costs of the biofilters were estimated to be

approximately 40% less than those for activated carbon adsorption and catalytic thermal oxidation (Hsu 2000).

The studies mentioned above demonstrate that the performance of VPBs can be acceptable in terms of overall VOC removal. However, the hydrophobic components of the VOC mixture seem to limit the overall removal achievable in a biofilter treating paint VOCs. Kazenski and Kinney (2000) demonstrated that surrogate paint VOC mixtures could be successfully degraded in a lab-scale biotrickling filter packed with polypropylene pall rings. Even though an overall removal efficiency of 94% was achieved, toluene and p-xylene removal was relatively poor (80% toluene and 60% xylene removal) (Kazenski 2000). Similar results were found in another study reported by Webster. A pilot-scale biotrickling filter was set up to treat off-gases from paint spray booths. Three different packing materials were tested: (1) a 50/50 (v/v) polyurethane foam/plastic random packing mix, (2) plastic random packing alone, and (3) a structured, straight-channel packing. The primary VOC contaminants were toluene, MEK, xylene, and n butyl-acetate (NBA). After a 5-day start-up period, MEK and NBA were removed with greater than 98% removal. However, toluene and xylene were removed less efficiently. Among the three media tested, the average contaminant removal efficiencies for toluene and xylene were 78% and 69%, respectively in the optimal packing material (a mixture of polyurethane foam cubes and random dump packing). For the worst packing material, a straight-channel packing, only 32% removal of toluene and 28% removal of xylene was achieved (Webster 1998). These studies indicate that biofiltration seems to be a promising technology but overall removal can be limited by the relatively poor removal of the aromatic compounds.

There are other practical challenges facing biofilters for paint spray booth applications. Paint booths typically operate 4 to 8 hours per day during the week and shutdown on weekends and holidays. These frequent shutdown and restarts add difficulty of employing biological treatment system for this application since microorganisms can suffer from substrate starvation. Another challenge is the variation in the composition of the off-gas stream from paint spray booth operations. Since a wide variety of paints, primers and thinners are used at maintenance facilities, the range of paint products used at even a single paint booth can be quite variable. For example, over a one-month period during 2004, painting personnel used six different paints, two different primers as well as a thinner product to paint vehicle and helicopter parts at a single paint booth at Ft. Hood, Texas (Ft. Hood, 2004). Although the composition and VOC content of each paint product varies, several VOCs are commonly found in the paints, primers and thinner at Department of Defense (DOD) facilities are presented in Table 2-4.

Table 2-4: Volatile organic compounds emitted from paint spray booths at DOD facilities [(1)].

acetone	methyl ethyl ketone
butanol	methyl isobutyl ketone
butyl acetate	methyl pentyl ketone
ethyl benzene	methyl propyl ketone
n-heptane	toluene
methyl acetate	ethyl 3-ethoxy propionate
methyl amyl ketone	*o,m,p*-xylenes
methyl cyclohexane	trimethybenzene

(1) Tyndall, 1999; Webster, 1998 a,b; Ft.Hood, 2001; Anniston, 2000

Because most painting operations are dynamic in nature (with paint guns frequently turned on and off as necessary), the concentration of VOCs in the waste gas exiting a paint booth can vary over an order of magnitude in a short period (i.e., within 15 minutes to an hour). The total VOC concentrations from conventional booths generally range from a few parts per million by volume (ppm_v) to 300 ppm_v (as carbon) (MSE Technology, 2002; Tyndall AFB, 1999; Webster 1998a, b). Thus, paint spray booths generate gas streams that are characterized not only by complicated mixtures but also by dynamically varying VOC concentrations. These properties of off-gas stream from paint spay booth increase the difficulty of treating paint emissions in biofilters.

2.2 BIOREACTOR PERFORMANCE-VOC REMOVAL

Bioreactor performance can be quantified and evaluated using several parameters. It would be preferable to choose a single parameter that would be easy to determine and would accurately predict bioreactor performance and the kinetics of biodegradation processes in vapor phase bioreactors. However, it is inevitable that several parameters are required to obtain an understanding of a biofilm system and biodegradation processes (Murphy *et al*., 1995). One of the most simple and widely used parameters used to evaluate bioreactor performance is overall pollutant removal efficiency (RE), which is defined as the fraction of the inlet pollutant removed in a biofilter. However, RE can vary with inlet pollutant concentration, air residence time, and microbial activity. In addition, RE is a function of operational period and biomass quantity in biofilters. Consequently, this parameter is most useful when comparing results obtained under a given operating condition in vapor phase bioreactors.

Pollutant elimination capacity (EC) is another commonly used parameter to assess bioreactor performance. EC is defined as follows:

$$EC = \frac{Q(C_{in} - C_{out})}{V} \quad (g\text{ - pollutant}/m^3\text{ - hr}) \qquad \text{(Equation 2-1)}$$

where, C_{in} is the inlet concentration (g/m^3), C_{out} is the outlet concentration (g/m^3), Q is the gas flow rate (m^3/hr), and V is the bioreactor volume (m^3).

EC curves are determined by sequentially increasing the inlet concentration stepwise for several hours until quasi-steady-state is reached (Deshusses and Johnson, 2000). Several VOC loadings are applied to a bioreactor, and the corresponding ECs are calculated using equation 2-1 above. Two parameters determined from the EC curves developed in this manner are commonly used as an indicator of bioreactor performance: (1) maximum EC, and (2) critical loading. Maximum EC is the point where an EC curve has its highest value, while critical loading is defined as the point at which the EC starts to deviate from the 100% removal line (Deshusses and Johnson, 2000). These two parameters are very useful to evaluate bioreactor performance under various conditions. However, it should be recognized that these parameters are not normalized by the quantity of microorganisms in a bioreactor. Therefore, the EC of a bioreactor varies with time of operation and is a function of biomass quantity, nutrient supply, and other operating parameters. Care should be given when interpreting EC data, particularly when biomass quantity significantly varies with operational period.

2.3 OTHER KEY OPERATING PARAMETERS

Bioreactor performance is controlled by not only substrate and microbial interactions but also by numerous design and operating parameters such as packing media

type, nutrient availability, pH, and moisture content. The following sections discuss these operating parameters.

2.3.1 Packing Media

Packing materials commonly used in biofilters include peat, compost or wood chips as well as inert materials such as ceramic pellets, activated carbon, and polyurethane foam. Organic packing materials may compact and degrade over time leading to a decline in performance and the need for periodic replacement. When inert synthetic packing materials are used, nutrients must be added regularly to the biofilter. Also, synthetic packing media is often more expensive than natural packing media. Nevertheless, inert packing media allows tighter control of key operating parameters such as nutrient delivery and biomass distribution and removal (Song 2000).

Whatever packing media is used, desirable packing media properties include high porosity, appropriate pore size, low density, and an ability to sorb water (Swanson and Loehr 1997). High porosity makes it possible to maximize the contact time between the gas stream and the biomass. Pore size is directly related to head loss and potential clogging problems in the biofilter as biomass growths develop. Low density is ideal for constructing biofilters and helpful for minimizing bed compaction due to packing material weight. The capacity to absorb water can be helpful for rewetting the packing media when drying problems occur in the biofilter. Finally, a key factor affecting practical application of biofilter media is cost. Even though several packing types have most of the desirable properties, the cost factor will limit practical application of expensive packing media. Moe (2000) revealed the potential advantage of polyurethane foam as a packing media. Depending on the manufacturing method employed, polyurethane foam can have various properties. Generally, the polyurethane foam has

many of the desirable properties mentioned above including high porosity, low density, and an ability to sorb water. In addition, excess biomass can be readily removed from foam packing materials via compression due to the sponge-like property of polyurethane foam media, which can prevent clogging over long-term operation in a biofilter (Moe 2000a; Moe 2000b).

2.3.2 Nutrients

Microorganisms require nutrients such as nitrogen, phosphorus, potassium, sulfur, and trace metals in addition to a carbon source to form new cell material. Among nutrient elements, nitrogen typically makes up 12 to 13% of dry cell mass, and phosphorus makes up 2 to 3% of the dry cell mass (Metcalf and Eddy 1991).

Several studies have been conducted to investigate the effect of nutrient limitation on bioreactor performance. Since nitrogen is one of the major cell components, it can possibly limit biomass growth if it is not supplied in sufficient quantities. Even though natural packing media such as compost, peat, soil, and wood bark have residual nutrients, additional nutrients are necessary to ensure reliable long-term performance of biofilters (Corsi 1995; Morgenroth 1996; Barton 1997; Gribbins 1998; Yang 2002).

The nitrogen that microorganisms can utilize is generally present in an inorganic form such as nitrate and ammonia. Organic forms of nitrogen are less available for microbial uptake (Gribbins 1998). Several studies have investigated the effectiveness of using these inorganic nitrogen sources to enhance biofilter performance. Several researchers have recommended nitrate as a nitrogen source for biofilter applications (Smith 1996; Jorio 2000; Yang 2002). Smith *et al*. (1996) demonstrated that ammonia as the sole nitrogen source resulted in the accumulation of nitrifying bacteria which have no known utility in biofilters and may interfere with the availability of ammonia nitrogen for

VOC-degrading microorganisms (Smith 1996). In another study, NH_3 at high concentrations had an inhibitory effect on methanol biodegradation while NO_3 at high concentrations did not affect the methanol removal rate (Yang 2002). Jorio *et al.* (2000) found that a biofilter supplied with ammonia as the nitrogen source had a higher styrene elimination capacity (up to 141g/m^3-hr) compared to the biofilter provided with nitrate (up to 50 g/m^3-hr). However, the use of ammonia for microbial growth resulted in higher biomass yields and resulted in the accumulation of excess biomass and clogging of the biofilter media (Jorio 2000).

In contrast to the results above, several other researchers have suggested using ammonia as the nitrogen source for microorganisms in biofilters. In a microcosm study, microorganisms had a higher toluene consumption rate at higher NH_3 concentrations even though toluene consumption was delayed in the media at higher nutrient concentrations during the initial acclimation period (Acuna 2002). Gribbins and Loehr (1998) demonstrated that ammonia might be a better choice as a nitrogen supplement when water was added biofilter media to counteract drying. Since ammonia is a cationic form of nitrogen, ammonia might resist leaching better than an anionic form, such as nitrate (Gribbins 1998). Ammonia may also have a strong binding capacity with bed material or organic matter in biofilm (Gribbins 1998; Yang 2002).

No standards have been established for nitrogen concentration and type in biofilter applications. It depends on the individual biofilter system. In one biofilter study, it was observed that as long as the biofilter had adequate amounts of microbially available nitrogen, additional nitrogen did not have a significant effect on biofilter performance (Gribbins 1998). Similar results were observed in another study by Holubar (1999). Biofilm growth responded strongly to the amount of available nitrogen, whereas hydrocarbon degradation efficiency reached a maximum of 60% and could not be

increased even by further addition of nitrogen. A different study of a compost biofilter treating toluene revealed that the biofilters' efficiency strongly depended on the nitrogen supply, and they suggested that the stoichiometric mass ratio (reactive carbon/reactive nitrogen) was 3.8 assuming that bacteria contained on average 13% of their mass as nitrogen and 50% as carbon (Delhomenie 2001). However, nutrients can become kinetically limiting in biofilters even though it may remain in stoichiometric excess. Rhin *et al.* (1997) demonstrated that mass transfer for components such as nitrate in the liquid phase could be limited by the liquid phase (Rihn 1997). Moe and Irvine (2001) also demonstrated that kinetic limitations caused a biofilter to fail even when a stoichiometric excess of nutrients was available (Moe 2001).

Nitrogen limitation has a greater effect on VOC degradation in a biofilter treating mixtures of compounds. In one study, increasing nitrogen availability resulted in sequential improvement of VOC removal in a biofilter treating a surrogate paint VOC mixtures containing methyl propyl ketone (MPK), n-butyl acetate (NBA), ethyl 3-ethoxy propionate (EEP), toluene, and xylene. At the lowest nitrogen availability, for example, NBA and EEP were completely degraded and only a portion of MPK was removed. With increasing nitrogen availability, MPK degradation increased and finally toluene and xylene removal improved (Song 2003). These results indicate that substrate interactions and microbial competition may be influenced by nutrient availability.

In addition to requiring nitrogen for microbial growth, minimal trace metals such as Chromium (Cr), Cobalt (Co), Copper (Cu), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Zinc (Zn), and Iron (Fe), and vitamin are also required. In one study, the addition of vitamins and trace metals during start-up period yielded a more stable and efficient biofilter, while addition of vitamin or trace metals to the macro-nutrient solution

later in the biofilter operation did not significantly improve biofilter performance (Pardo 2002).

2.3.3 pH

The optimal pH of the aqueous phase should be between 6 and 9 in most microbial bioreactor systems treating volatile organic compounds (Leson 1991; Kinney 1999; Madigan 2000). If the biofiltration process leads to acidic end products like hydrochloric acid, nitric acid or sulfuric acid that can lower bed pH and decrease removal efficiency, the bioreactor should be well buffered (Neal 1998). Fungi overgrow the bacteria at a low pH, which may affect the performance of the bioreactor (van Groenestijin 1994; Sanhez-pena 2000). In order to control pH in a biofiltration system, the addition of buffers in the form of phosphate, lime or ammonium hydroxide should be considered.

2.3.4 Moisture Content

One of the most important parameters affecting biofilter performance is the moisture content of the packing material. A non-optimum moisture content can lead to compaction, breakthrough of incompletely treated waste gas and the formation of anaerobic zones which emit odorous compounds (Leson 1991). Poor humidity control leads to drying and poor pollutant elimination capacity in biofilters. Deshusses and Johnson (1999) demonstrated that the failure of a pre-humidification system caused significant reduction in the moisture content of the packing material. Even though the initial reduction of moisture content did not immediately affect performance, further dry-out caused rapid and nearly complete loss of biological activity and ethyl acetate

reduction after 3 days (Deshusses 1999). Moisture contents of 40 to 60 % by weight should be maintained for optimal biofilter operation (Ottengraf 1986). Depending on the packing media used in a biofilter, slightly different optimal moisture contents are required. Moe and Irvine (2000) suggested 65 % moisture content for the hydrophilic polyurethane foam packing media (Moe 2000b).

2.4 BIODEGRADATION OF POLLUTANT MIXTURES

Industrial waste gases are usually polluted with more than one compound. Aircraft and automotive paint spray operations generate waste gas streams containing a complex mixture of readily degradable and relatively recalcitrant compounds. Thus, substrate competition or inhibition may occur during the biodegradation of these mixtures in biofilters. This may lead to an undegraded target compound or unacceptably large installations. These problems may limit the practical applicability of this intrinsically environmental friendly technique (Okkerse 1999). Even though BTEX compound interactions have been studied extensively for pure and mixed microbial species, few studies have been completed to investigate substrate interactions that may occur with paint VOC mixtures. In order to apply biofiltration technology to the treatment of off-gas streams from paint spray operations, a better understanding of the effect of VOC mixtures on pollutant degradation profiles in biofilters is needed.

In this section, the basic types of multiple substrate degradation patterns and positive or negative effect of multiple substrate interactions are presented. The effects of microbial acclimation on observed substrate interactions are also described. In addition, studies of BTEX compound interaction are summarized as a well-studied example of multiple substrate interactions.

2.4.1 Multiple Substrate Degradation

The biodegradation of multiple compounds can be categorized as diauxie, co-metabolism, simultaneous degradation, or competitive inhibition (Madigan 2000). Diauxic growth or sequential degradation results from catabolite repression or inhibition where an organism grows on one carbon source and then the other. Thus growth occurs as two exponential phases separated by a distinct lag phase. In the case of diverse mixed cultures growing on binary substrate mixtures, diauxic growth is not expected, but in some cases, either it or some other form of sequential carbon substrate utilization occurs (Madigan 2000; Hamer 2001). Co-metabolism refers to the metabolic degradation of a substance while a second substance serves as primary energy of carbon source. Utilization of the co-metabolized carbon source does not result in biomass growth (Madigan 2000; Zheng 2001). When multiple compounds can be degraded at the same time, it is called simultaneous degradation. Another common type of substrate interaction, competitive inhibition, may also exist during multiple substrate degradation. Competitive inhibition can be either a competition between bacterial communities or substrate competition. Different communities are specialized for the degradation of different compounds. For example, one may be specialized for the elimination of oxygenated compounds, while the others are more specialized for elimination of aromatic and halogenated compounds. When substrate competition dominates, the bacterial community is relatively constant over the height of the bioreactor column, but the more easily biodegradable compounds are used first for microbial metabolism (Aizpuru 2001).

Positive/Negative effects of multiple substrate interactions

The presence of easily degradable compounds can have a positive or negative effect on the degradation of multiple substrates. As a positive effect, the presence of an

easily biodegradable substrate like ethanol increases biomass. In one study, the presence of ethanol significantly increased o-DCB (ortho-dichlorobenzene) removal rates (Bhattacharya 2001). Similar findings were observed in BTEX degradation in a bottle. Even though the presence of ethanol decreased the metabolic flux of toluene, this negative effect was counteracted by ethanol-supported biomass. Eventually, the increased biomass quantity resulted in faster degradation rates of toluene. Thus, its positive effect on cell growth outweighed its negative effect on the metabolic flux for benzene at low ethanol concentrations. However, high ethanol concentrations had a negative effect on toluene and benzene degradation because it caused oxygen limitations (Lovanh 2002). Deeb *et al*. (2001) studied substrate interaction in a pure culture, PM1 which is capable of utilizing MTBE for growth. PM 1 was able to degrade benzene and toluene as a single carbon source. However, when MTBE-grown cells of PM 1 were exposed to MTBE/benzene and MTBE/toluene mixtures, MTBE degradation proceeded, while the degradation of benzene and toluene was delayed for several hours. Following this initial lag, benzene and toluene were degraded rapidly, while the rate of MTBE degradation slowed significantly. MTBE degradation did not increase to the previous rate until benzene and toluene were almost entirely degraded. They suggested that the lag in benzene and toluene was probably due to the induction of the enzymes necessary for BTEX degradation. Also the energy generated during the degradation of MTBE mixture with benzene initially allows for the efficient production of the enzymes needed for benzene degradation. That's the reason why the presence of MTBE, pyruvate, or ethanol enhanced the benzene degradation compared to benzene degradation alone (Deeb 2001).

In contrast to the positive effects of easily degradable substrates in a mixture, Mohseni and Allen (2000) demonstrated that the presence of methanol, a hydrophilic and easily biodegradable compound, suppressed the growth of the α-pinene degrading

community, thereby reducing α-pinene removal capacity in the biofilters. Unlike α-pinene, methanol was not affected by the presence of α-pinene and was removed at the same rate regardless of the α-pinene loading rate (Mohseni 2000). Methanol did not directly inhibit the α-pinene-degrading community, but results suggest that eventually a methanol-degrading community dominated the biofilter. The removal rate of α-pinene gradually decreased along the column after each step increase in methanol inlet concentration. It appears that the presence of high concentrations of methanol, a hydrophilic and easily degradable compound, adversely impacted the growth of the α-pinene degrading microbial community, thereby decreasing the α-pinene removal capacity of the biofilters over time (Mohseni 1999). Similar results were observed in a toluene treating biofilter subjected to high loads of ethyl acetate. Despite the presence of toluene degraders, the removal of toluene was inhibited by high loads of ethyl acetate. Toluene removal did not commence until the ethyl acetate concentration dropped to less than 0.5 g/m^3. Plate counting of the microbial population in the biofilter indicated that toluene degraders were present in the non-toluene degrading segment of the biofilter and that they were able to degrade the toluene transferred from the gas phase to the biofilm. In addition, all of the isolated toluene degraders were able to degrade ethyl acetate.

The positive or negative effects of substrate interactions vary with each substrate concentration and microbial species involving in the degradation. The complexity increases as the number of substrates involved in the biodegradation process increase. When *Pseudomonas putida* was grown on a mixture of carbazole, *p*-cresol and sodium salicylate, strong interactions were observed. Even though this bacterium could not utilize carbazole as a sole carbon and energy source, sodium salicylate not only supported cell growth but was also responsible for inducing some specific enzymes for carbazole degradation. Carbazole degradation started only after *p*-cresol was significantly or

completely removed, and the removal of carbazole was incomplete when the initial *p*-cresol concentration was higher than 20 mg/L. No carbazole was removed at all when the initial *p*-cresol concentration in the system was higher than 120 mg/L (Yu 2002).

In another study, the ability of a single pure culture, *Mycobacterium vaccae* to degrade groundwater pollutants was investigated in sealed vials. When toluene and benzene were both present, toluene was catabolized and benzene oxidation was delayed. Although toluene promoted the degradation of styrene, a lower rate of toluene degradation occurred when styrene was present (Burback 1993). Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates have also been investigated (Reardon 2000). The substrates were catabolized by the same enzymatic pathway, but purely competitive kinetics did not explain the substrate interactions well. Toluene significantly inhibited the biodegradation rate of the other substrates, and benzene slowed the degradation rate of phenol but not of toluene. Phenol had little effect on the biodegradation of either toluene or benzene (Reardon 2000). These studies indicate that substrate interactions are likely system-dependent and can have both positive and negative effects on the rates of pollutant degradation.

Effect of microbial acclimation on substrate interactions

Substrate interactions can be different even in the same microbial culture depending on how the microbial culture was initially acclimated. The biodegradation of resorcinol and catechol was studied in upflow anaerobic fixed film-fixed bed reactors (Swaminathan 1999). Cross feeding studies revealed that phenol was poorly degraded in the resorcinol acclimated reactors whereas it was readily degraded in catechol acclimated reactors. The catechol culture seemed to be the most versatile in degrading phenol, resorcinol and hydroquinone. Resorcinol cultures exhibited rigid substrate specificity

whereas the catechol acclimated culture seemed to have relaxed substrate specificity for phenol as well as for its isomers. Another study revealed similar results. When a mixed substrate (phenol and glucose) was used, the phenol acclimated population (*Arthrobacter* species) showed an initial preference for phenol and utilized glucose after phenol removal. However, the phenol degradation rate was reduced in the presence of glucose. The inoculum from a mixed substrate acclimated culture showed immediate degradation of both glucose and phenol (Kar 1996). These findings suggest the importance of acclimation history but did not address whether the microbial community had changed during the acclimation process.

BTEX compound biodegradation

As a well-studied example of multiple substrate degradation, the studies of BTEX compound interaction are summarized below. Relatively extensive studies of BTEX biodegradation have been conducted over the last 10 years. Results of these studies indicate that the substrate interactions between BTEX compounds often vary with microbial culture and culture conditions. As a result, reported results have yielded conflicting conclusions regarding microbial activity toward BTEX compounds.

Alvarez and Vogel (1991) studied BTX compound degradation by an indigenous mixed culture in sandy aquifer material and by a pure culture of *Pseudomonas sp*. Strain CFS-215 and *Arthrobacter sp*. strain HCB respectively. Beneficial substrate interactions included enhanced degradation of benzene and p-xylene in the presence of toluene in the *Pseudomonas sp*. Strain CFS-215 incubations, as well as benzene-dependent degradation of toluene and *p*-xylene by *Arthrobacter sp*. strain HCB. Detrimental substrate interactions included retardation of benzene and toluene degradation in the presence of *p*-xylene in both aquifer slurries and *Pseudomonas* incubations (Alvarez 1991).

Two *Pseudomonas* species (strains B1 and X1) were tested for BTEX biodegradation in another study by Chang. Strain B1 grew with benzene and toluene as sole sources of carbon and energy, and it cometabolized *p*-xylene in the presence of toluene. Strain X1 grew on toluene and *p*-xylene, but not benzene (Chang 1992).

A microbial consortium and *Pseudomonas* strain (PPO1) was used to study the biodegradation of benzene, toluene, and *p*-xylene. Benzene and toluene mixtures were removed under cross-inhibitory (competitive inhibition) kinetics in both cultures. In the presence of benzene and/or toluene, p-xylene was cometabolically utilized by both cultures, but was not completely mineralized (Oh 1994).

Yerushalmi and Guiot (1998) studied the biodegradation of gasoline. Benzene degradation was initially suppressed by substrate interactions, but proceeded at an increased rate when the respective concentrations of toluene and ethylbenzene were low. The presence of competitive inhibition among the gasoline hydrocarbons is due to the fact that these hydrocarbons have similar structures and share many enzymes in the biochemical pathways followed during their respective degradation (Yerushalmi 1998).

A substrate interaction study of BTEX compounds was conducted in a microbial consortium which was derived from a gasoline-contaminated aquifer, and also in a pure strain, *Rhodococcus rhodochrous*. For a single compound in both pure and mixed cultures, toluene was degraded fastest, followed by benzene, ethyl benzene, and the xylenes. In BTEX mixtures, degradation followed the order of ethyl benzene, toluene, and benzene, with the xylenes degraded last (Deeb 1999).

Toluene was found to enhance the catalytic efficiency of a biofilter for p-xylene, while catabolism of all the other BEX compounds was inhibited competitively by the presence of toluene. The toluene-acclimatized biofilter was able to degrade all of the other BEX compounds, even in the absence of toluene (du Plessis 2001).

Lee *et al*.(2002) found that while xylene could not be utilized as a growth substrate by *Stenotrophomonas maltophilia* T3-c, the presence of toluene resulted in the cometabolic degradation of xylene. The specific degradation rate of toluene was increased by the presence of benzene, ethylbenzene, or xylene in binary mixtures. The presence of toluene or xylene in binary mixtures with benzene increased the specific degradation rate of benzene. The presence of ethylbenzene in binary mixtures with benzene inhibited benzene degradation. The presence of more than three kinds of substrates inhibited the specific degradation rate of benzene (Lee 2002).

The soil fungus *Cladophialophora* sp. strain T1 was investigated for the biodegradation of BTEX compounds. Benzene was not metabolized, but the alkylated benzenes (toluene, ethylbenzene, and xylenes) were degraded by a combination of assimilation and cometabolism. Toluene and ethylbenzene were used as sources of carbon and energy, whereas the xylenes were cometabolized to different extents. o-Xylene and m-xylene were converted to phthalates as end metabolites; p-xylene was not degraded in complex BTEX mixtures but, in combination with toluene, appeared to be mineralized (Prenafeta-Boldu 2002).

Under anaerobic conditions, the biodegradation of jet fuel compounds such as toluene, o-xylene, 1,2,4-trimethylbenzene(TMB), and naphthalene, separately or together was investigated. Toluene and o-xylene were degraded both as a single substrate and in a mixture with the other aromatic compounds, while TMB was not biodegraded as a single substrate, but was biodegraded in the presence of the other aromatic hydrocarbons (Zheng 2001).

As summarized above, substrate interactions vary in microbial systems as a function of the substrate mixture composition, microbial species, and acclimation

method. To get a better understanding of substrate interactions in a specific system, all these parameters should be considered.

2.4.2 Microorganisms

Bioreactor performance is controlled by several factors such as substrate competition, physiological properties of the microbial community, and other operating parameters (Fernandez 2000). Several studies have been done to investigate the effect of microbial community structure on bioreactor performance and microbial population shifts during bioreactor operation (Stoffels 1998; Fernandez 1999; Fernandez 2000; Tresse 2002; Haruta 2002a; Haruta 2002b).

A complex community can achieve high degradation efficiency and stability for degrading natural cellulosic materials such as rice straw (Haruta 2002a), and an extremely dynamic community can maintain stable ecosystem function (Fernandez 1999). In contrast, Haruta *et al.* (2002b) suggested that low diversity was useful for maintaining the stability of the community structure and function. In their study, only a few species constituted most of the total eubacterial cells during the biodegradation of multiple substrates, such as those in household garbage(Haruta 2002b).

Microbial communities treating even single chemicals in a biofilter contain dozens of interacting microbial species. During biotrickling filter operation for styrene removal, population dynamics was analyzed using a denaturing gradient gel electrophoresis (DGGE) method (Tresse 2002). They found that the biofilm community was more complex than the suspended cell community. In addition, only 50 % of the bands representing the inoculum were present in the biomass established within the biotrickling filter. This result suggests that the biomass established in the column was subjected to further enrichment as compared to the inoculum culture, even though the

inoculum was adapted to styrene. Stoffels (1998) observed two significant shifts in the bacterial community structure in a trickle-bed bioreactor using fluorescent in situ hybridization (FISH). One significant shift occurred followed the transfer of the original inoculum from the wastewater to the fermenter. Another significant shift was observed when the fermenter culture was transferred to the trickle-bed bioreactor (Stoffels 1998). However, since the FISH technique only targets microbial species with already known 16S rRNA, the microbial community shift can not be fully observed using this technique. In another study, *Pseudomonas putida*, *Pseudomonas putida* biotype A, *Rhodococcus* sp., and *Arthrobacter paraffineus* were individually incubated with toluene and mixed before biofilter inoculation (Jorio 1998). When toluene and xylene were present together, toluene biodegradation was inhibited by the presence of xylene in the biofilter. Several weeks after start-up, none of the inoculating strains remained dominant in the biofilm. However, all strains isolated from the biofilm were positive in at least one physiological property related to the degradation of aromatic organic molecules (Jorio 1998). The authors relied on the microscopic morphology features and several physiological tests to compare the inoculating strains to the ten dominant strains found in the biofilter several weeks after biofilter start up. This technique relies on cell culturing and again may not properly represent the microbial community.

The microbial community in a bioreactor is also apt to change when the operating conditions change. For instance, in a soil bioreactor treating 3-chlorobenzoate, feed modification led to microbial community changes that were observed using the DGGE method (Fantroussi 1999). Nakagawa *et al.* (2002) observed successive changes in DGGE band profiles of an ethylbenzene-degrading sulfate-reducing consortium. In their study, the dominant population in this consortium decreased when the ethylbenzene concentration in the carrier phase decreased accompanied by the production of sulfide. In

addition, when the enrichment was transferred into a different substrate medium, the dominant band disappeared and different bands appeared for all tested substrates (Nakagawa 2002). In another study, changes in the microbial community during carbohydrate fermentation were detected at various dilution rates by DGGE analysis, and these changes corresponded to the changes observed in product distributions (Ueno 2001). Sun *et al.* (2002) observed microbial community changes in a biofilter with the varying moisture content in the packing material. In a biofilter treating toluene, the quantity of molds and actinomyces decreased and the number of bacteria increased when the initial moisture content was high (Sun 2002). To date, no DGGE studies have been completed to monitor microbial population shifts that may occur in biofilters due to different acclimation methods or as a function of operating time.

2.5 MICROBIAL MONITORING TECHNIQUES

The microbial community found in engineered bioprocesses has traditionally been monitored using most probable number (MPN) or total heterotrophic plate counts. However, these cultivation techniques yield only a small culturable fraction (0.1 – 10%) of the organisms present in environmental samples. Hence, conventional techniques that depend on cell culturing are not representative of the microbial community of complex systems and they tend to underestimate both the microbial population number and diversity (Castro 1997; Head *et al*., 1998).

Recently, the application of molecular biological methods to study the diversity and ecology of microorganisms in natural environments has been practiced. Due to the ubiquity of ribosomal RNA molecules (16S and 23S, in Prokaryotes; 18S and 28S, in Eukaryotes) in all cellular life forms, comparative analysis of their sequences can be universally applied to infer relationships among organisms. The rRNA molecules

comprise highly conserved sequence domains interspersed with more variable regions. In general, essential rRNA domains are conserved across all phylogenetic domains, thus "universal" tracts of sequences can be identified (Madigan *et al*., 2000; Head *et al*., 1998). With these molecular techniques which involve analysis of ribosomal RNA sequences, many new insights into the composition of uncultivated microbial communities have been gained. Three molecular techniques that have been widely used are discussed as follows:

FAME (Fatty Acid Methyl Ester) analysis is a technique which identifies organisms based on their cellular and membrane fatty acids. Since the types and composition of fatty acids present in bacteria are species specific, fatty acids are used for microbial identification. The technique of FAME analysis is based on the methylation and extraction of microbial fatty acids which are then rapidly analyzed by gas chromatography (Castro 1997). However, since FAME analysis uses cellular components for analysis, it has been noted that when applied to complex communities, there is limited meaningfulness in the results (Haack 1994). Variation in organism fatty acid composition, due to environmental conditions, and the potential interference from organic materials in samples, has not been thoroughly considered and may complicate the interpretation of the results (Castro 1997).

FISH (Fluorescence In Situ Hybridization) has been used successfully for studies in microbial ecology (Stoffels 1998). Nucleic acid probes can be designed that will react only with cells of bacteria, archaea or eukarya because of unique signature in their ribosomal RNA. The binding of probes to cellular ribosomes can be seen microscopically when a fluorescent dye is attached. By treating cells with the appropriate reagents they

become permeable and allow penetration of the oligonucleotide probe/dye mixture. Following hybridization of the probe directly to the ribosomal RNA in the cell's ribosome, the cells become uniformly fluorescent and can be observed under a fluorescent microscope. This technique can be applied directly to cells in culture or in a natural environment (Madigan 2000). However, the FISH technique only targets bacterial species with already known 16S rRNA sequences (Tresse 2002). It is also restricted to active cells, which could be a drawback in biofilm studies as biofilm cells have different levels of activity (Amann R I. 1995).

DGGE (Denaturing Gradient Gel Electrophoresis) is a method based on the electrophoretic separation of PCR-amplified ribosomal gene fragments with the same length but with different sequences on a linear denaturing gradient polyacrylamide gel (Tresse 2002; Zhang and Fang, 2000). Individual bands may be excised, reamplified and sequenced, or challenged with a range of oligonucleotide probes, to give an indication of the composition and diversity of the microbial community. DGGE banding patterns can be numerically analyzed without further sequencing when detailed identifications of microbial communities are not necessary. For example, Tresse *et al.* (2002) used the Sorenson index (Cs) to numerically analyze the spatial distribution of a microbial population in a biotrickling filter (Tresse 2002). The dissimilarity index, D and Shannon-Weaver Index, H have been used in other studies to quantify the difference between communities and estimate the structural diversity of the microbial community (Luxmy 2000). Generally, DGGE is relatively rapid to perform, and many samples can be run simultaneously. In our study, this DGGE method will be used to investigate how the microbial population shifts in the biofilter over time and as a function of nitrogen availability. The remainder of this section provides an overview of the DGGE analysis.

The DGGE technique can be used to identify single base changes in a segment of DNA. The extracted DNA from a microbial sample is subjected to PCR amplification using "universal" primers or primers designed to amplify rRNA genes from a particular group of organisms. In a denaturing gradient acrylamide gel, double-stranded DNA is subjected to an increasing denaturant environment and will melt in discrete segments called "melting domains": stretches of base-pairs with an identical melting temperature. The melting temperature (T_m) of these domains is sequence-specific. When a domain with the lowest melting temperature reaches its melting temperature (T_m) at a particular position in the denaturing or temperature gradient gel, the DNA will become partially melted, creating branched molecules. Partial melting of the DNA reduces its mobility in a polyacrylamide gel. Sequence variation within such domains causes the melting temperatures to differ, and molecules with different sequences will stop migrating at different positions in the gel. If the fragments completely denatures, then migration again becomes a function of size. However, optimal resolution is attained when the molecules do not completely denature and the region screened is in the lowest melting domain. To achieve this, a 30-40 base pair GC clamp is added to the one of the PCR primers, coamplified and thus introduced into the amplified DNA fragments (Sheffield *et al*., 1989; Sheffield *et al*., 1992). The GC-rich sequence act as a high melting domain preventing the two DNA strands from complete dissociation into single strands (Myers *et al*., 1985). In DGGE, the denaturing environment is created by a combination of uniform temperature, typically between 50 and 65^{o}C and a linear denaturant gradient formed with urea and formamide. A solution of 100% chemical denaturant consists of 7 M urea and 40% formamide. The denaturing gradient may be formed perpendicular or parallel to the direction of electrophoresis. A perpendicular gradient gel, in which the gradient is

perpendicular to the electric field, typically uses a broad denaturing gradient range, such as 0-100% or 20-70% (Fisher and Lerman, 1983). In parallel DGGE, the denaturing gradient is parallel to the electric field, and the range of denaturant is narrowed to allow better separation of fragments (Myers *et al*., 1987).

DGGE of PCR-amplified rDNA fragments has been used to provide information on the genetic diversity of microbial communities and to profile community complexity of a microbial mat and bacterial biofilms (Muyzer *et al*., 1993; Muyzer *et al*., 1995; Moyer *et al*., 1994, 1995). This DGGE technique has been applied to study community changes as well. Since microbial ecological studies often require sampling at different time points over a long period, cloning techniques are not suited for the analysis of many different samples. By using DGGE, many samples taken at different time intervals during the study can be simultaneously analyzed (Donner *et al*., 1996; Santegoeds *et al*., 1997; Teske *et al*., 1996). The method is, therefore, particularly useful when examining time series and population dynamics. Once the identity of an organism associated with any particular band has been determined, fluctuation in individual components of a microbial population, due to environmental perturbations, can be assessed. This technique is therefore well suited for monitoring the dynamics of mixed populations of microorganisms (Head 1998).

Chapter 3 Baseline Biofilter Experiments

Most biofiltration studies to date have focused on the treatment of a single pollutant in order to minimize the complexity of the systems and to elucidate the effect of operating parameters on biofilter performance. However, industrial waste gases are usually polluted with more than one compound. Aircraft and automotive paint spray operations generate a complex mixture of readily degradable and relatively recalcitrant compounds. Thus, substrate competition or inhibition may occur during the biodegradation of these mixtures in biofilters. Theses substrate interactions in a mixture are more likely system-dependent and can have both positive and negative effects on the rates of pollutant degradation. For example, over the last 10 years, relatively extensive studies of BTEX biodegradation have been conducted. The results of these studies indicate that the substrate interactions between these BTEX compounds often vary with microbial culture and operating conditions, which have often yielded conflicting conclusions.

Thus, in order to apply biofiltration to paint spray emissions, it is necessary to identify key operating parameters affecting the performance of the biofilter and develop strategies to overcome these limitations. To simplify the experimental system, five components found in the highest fraction in an operating automotive paint spray booth were selected as a representative paint VOC mixture for this task (Wander 1999; Anniston, 2000; Ft.Hood, 2001). A previous study conducted by Kazenski (2000) indicated that a biotrickling filter packed with polypropylene pall rings could achieve VOC removals as high as 94% when treating this surrogate paint mixture. In this experimental task, a biofilter packed with polyurethane foam media was set up to treat surrogate paint mixtures (see Figure 3-1 below)

Two sets of biofilter experiments were conducted in this phase of the research to investigate the biodegradation patterns of surrogate paint VOC mixtures in a biofilter. During the first set of experiments, the factors controlling the removal of a paint VOC mixture were identified. Then, during the second set of biofilter experiments, a new operating approach was investigated for its ability to overcome limitations identified in the first set of experiments. The specific hypotheses addressed in this phase of the research are as follows:

- Since paint spray booths generate a complex mixture of both hydrophilic and hydrophobic compounds as well as readily degradable and relatively less degradable compounds, hydrophobic compounds and relatively less degradable compounds may limit the overall removal efficiency achievable in the system.
- Nutrient availability may be a crucial factor affecting the establishment of an effective biofilm in the biofilter.
- The VOC degradation profiles along the biofilter may vary depending on the operating conditions in the system. Growth conditions in biofilters are significantly different from those found in well-mixed liquid batch systems. Thus, changes in the environmental conditions within a biofilter may affect the microbial community in the biofilters, which may, in turn, result in different VOC degradation patterns.

3.1 EXPERIMENTAL METHODS

3.1.1 Bench Scale Bioreactor Design

The bench scale biofilter column used in this study was constructed from stainless steel and had an inner diameter of 16.2 cm. The biofilter column consisted of three individual sections that were bolted together (Figure 3-1). Each section was packed with polyurethane foam cubes (1.5 cm) to a height of 19 cm. A 12 cm plenum was located between each packed section to allow for gas sampling and redistribution of the contaminant stream between sections. A nutrient solution was periodically sprayed over the top of the column at 2L/min. The spraying frequency and composition of the nutrient medium were altered to optimized bioreactor performance as discussed in Section 3.1.5 below.

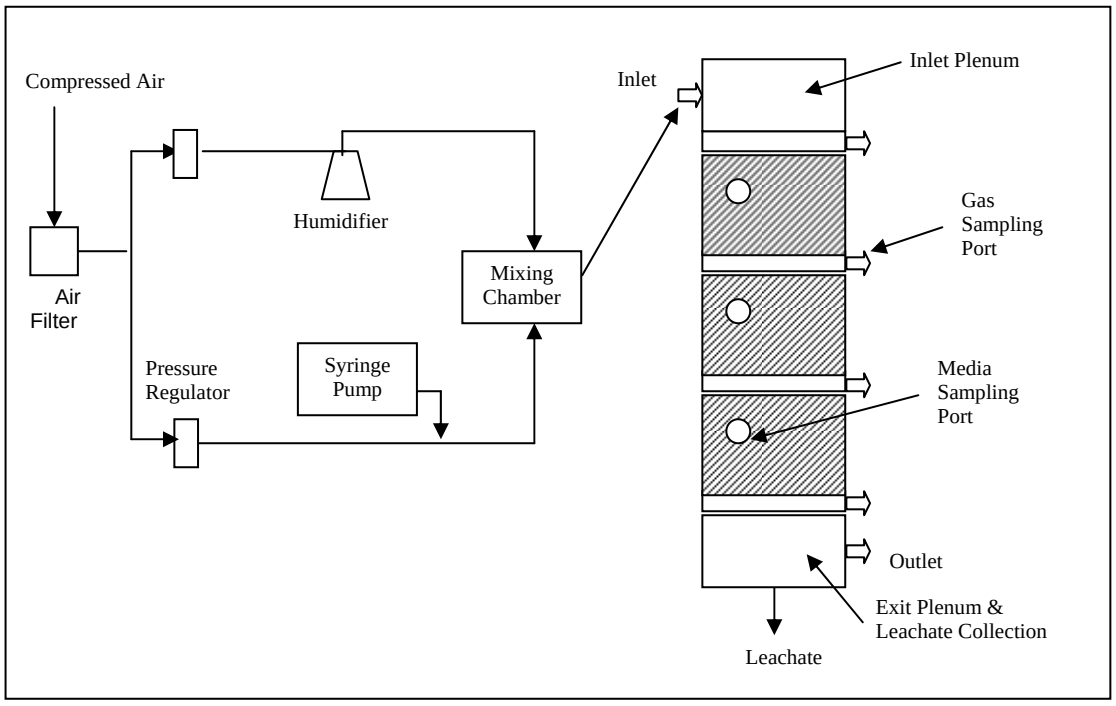


Figure 3-1: Bench Scale Biofilter Design.

3.1.2 Surrogate Paint Mixture for the Lab-Scale Experiments

Paint spray booth emissions contain a wide variety of VOCs which will vary with the formulation of the paint(s) being used. Nevertheless, several classes of compounds are generally found in paint emissions as evident in Table 2-4. Five components found in the highest fraction in an operating automotive paint spray booth were selected as a representative paint VOC mixture (Wander 1999; Anniston, 2000 ; Ft.Hood, 2001) for the lab-scale experiments. This surrogate paint mixture consisted of methyl propyl ketone (MPK), n-butyl acetate (NBA), ethyl 3-ethoxy propionate, toluene, and *p*-xylene. Properties of the surrogate paint VOCs examined in this study are presented in Table 3-1.

Table 3-1: Properties of the surrogate paint VOC mixture used in the lab-scale experiments.(1)

Compound	Formula	Molecular Weight (g/mol)	Density (g/ml)	Henry's Constant (atm/mol-m^3)	Solubility (g/100ml)
Methyl n-propyl ketone (MPK)	$C_5H_{10}O$	86.13	0.8095	$7.2929*10^{-5}$	4.3
n-butyl acetate (NBA)	$C_6H_{12}O_2$	116.16	0.8820	$2.5732*10^{-4}$	0.68
Ethyl 3-ethoxy propionate (EEP)	$C_7H_{14}O_3$	146.19	0.9500	$6.4991*10^{-4}$	2.9
Toluene	C_7H_8	92.15	0.8996	$6.3522*10^{-3}$	0.0526
p-xylene	C_8H_{10}	100.17	0.8660	$6.1547*10^{-3}$	0.0175

(1) Chemfinder 2003

As can be seen in Table 3-1, the surrogate paint mixture contains three hydrophilic and two hydrophobic compounds. The target inlet concentration for each compound is summarized in Table 3-2.

Table 3-2: Target Inlet VOC Concentrations.

VOC compound	Inlet concentration (ppm_v)
Methyl n-propyl ketone (MPK)	110
n-butyl acetate (NBA)	20
Ethyl 3-ethoxy propionate (EEP)	32
Toluene	20
p-xylene	18
Total	200

3.1.3 Analytical Methods

VOC Gas Measurements

Gas samples were periodically collected from each gas sampling port in the lab and pilot scale bioreactors with 0.5-mL gas-tight syringes fitted with a Mininert valve and a side bore needle (Hamilton 1700 series). The samples were analyzed using a Hewlett Packard Model 6890 Gas Chromatography (GC) equipped with a flame-ionization detector (FID) and a HP-5 capillary column. A HP-5 column was used with He as a carrier gas at a flow rate of 1.8 mL/min. The make-up gas flow to the detector was consisted of He (28.4 mL/min), H_2 (35mL/min) and zero grade air (350 mL/min). The column temperature started at 60 ^oC for 4 min and increased at the rate of 60 ^oC/min to 180 ^oC. The injector and detector temperatures were maintained at 200 ^oC. The GC was calibrated using five gas standards with known concentration of each chemical to be

analyzed. A 0.5-mL sample of each standard was injected onto the GC, and a five-point calibration curve was produced. Calibration curves were determined for each of the five chemicals in the inlet gas stream.

CO_2

CO_2 evolution from the bioreactor was monitored with a CO_2 analyzer (LI-COR, Model LI-6252). Gas sampling ports along the column were directly connected to the CO_2 analyzer with Teflon tubing. The digital output reading on the analyzer was allowed to stabilize and then a final measurement was taken.

Moisture Content

To determine the moisture content in the vapor phase bioreactor, packing media samples were collected from each sampling port. A ceramic weighing dish was first washed, and then dried overnight in a 105 ^{o}C oven. The dish was cooled to room temperature in a desiccator containing Drierite. The dish was then weighed on a balance with 0.1 mg accuracy (Ainsworth, Model M-220). The weight was then retaken after three pellets were placed in the dish. The dish and packing media were dried overnight. The dry dish and packing media were then placed in the desiccator and allowed to cool to room temperature before being reweighed. The moisture was calculated on a dry weight basis as follows:

$$Moisture\ Content = \frac{weight\ of\ water}{weight\ of\ dry\ packing\ media} \qquad \text{(Equation 3-1)}$$

Pressure Drop

The pressure drop across the column was measured periodically by connecting a pressure gauge (Magnehelic, Model 2001C and Magnehelic, Model 2005C) to the sampling ports located on the top and bottom of the bioreactor. The pressure difference was measured in inches of H_2O.

pH

The pH of leachate drained from the bioreactor column was monitored using an Accumet ® pH meter (Fisher Scientific, Model 50, Houston, TX). An automatic temperature correction (ATC) probe was used while measurements were being taken. The probe was standardized by autorecognition of U.S. Standard buffers at pH 7 and 10.

COD

The COD of biofilm samples collected from the experimental bioreactors was measured to monitor biomass accumulation in the bioreactors. Two packing media samples were removed from each section and placed in a 50 mL vial. The vials were filled with 40 mL of Ultra High Purity (UHP) water. Biomass was removed from the packing material using a sonification device (FS6 Fisher Scientific Sonicator) and a vortex mixer. Once all the biomass was removed from the packing media, a 2 mL sample from the well-mixed vial was drawn off and added to a COD vial. After mixing, the COD vials were placed in COD heating blocks and heated at 150 ^{o}C for 2 hrs. The COD vials were cooled down to room temperature before measuring absorbance at a wavelength of 690 nm in a spectrophotometer (Turner Model 690). The COD analyses complied with the Hach Method 8000 for COD determinations through colorimetric measurements (The

Hach Company, 1997). For the COD standard curve, six standard solutions ranging from 0 to 2000 mg/L COD were prepared and analyzed. The standard solution was made using a potassium hydrogen phthalate solution prepared according to standard methods (American Public Health Association 1992).

Inorganic Nitrogen (ammonia & nitrate nitrogen)

Samples for ammonium and nitrate analysis were prepared by adding the appropriate amount of biofilm sample to 10 mL of deionized water. The samples were homogenized by a 1 minute vortexing and 5 minutes sonication. The liquid ammonium concentration was determined with an ammonium electrode (ORION®, Model 95-12, Boston, MA). Nitrate measurements were performed in a similar manner using a nitrate combination electrode (Accumet, Fisher Scientific, NJ). The electrode probes were calibrated with 6-point NH_4Cl and KNO_3 standard solutions. To adjust ionic strength for both samples and standards, 0.4 mL of the ionic strength adjustment (ISA) solution (Fisher Scientific, NJ) was added for the ammonium measurements, while 0.4 mL of 2M $(NH_4)_2SO_4$ was added for the nitrate analyses. The electrode probes were allowed five to ten minutes to stabilize before reading each sample. Five-point standard curves for both ammonium and nitrate were used to calibrate the electrode responses.

3.1.4 Inoculation

The inoculum solution for the biofilters used in the baseline experiments was seeded with leachate from another bioreactor operating in our laboratory treating a mixture of paint VOCs (Kazenski 2000). The bioreactor inoculation culture was grown

at 23°C in 250-mL glass Boston round bottles containing 100 mL of nutrient medium (Table 3-3).

Table 3-3: Composition of Nutrient Medium[1]

Nutrient Media (g/L)		Trace nutrient solution [2] (mg/L)	
KH_2PO_4,	1.36	$MgSO_4 \cdot 7H_20$	0.05
Na_2HPO_4,	0.71	$CaCl_2 \cdot 2H_2O$	0.0147
KNO_3,	3.13	H_3BO_3	2.86
$(NH_4)_2HPO_4$,	0.66	$MnSO_4 \cdot H_2O$	1.54
trace nutrient solution[2]	1 mL/L	$FeSO_4 \cdot 7H_2O$	2.50
		$CuCl_2 \cdot 2H_2O$	0.027
		$ZnSO_4 \cdot 7H_2O$	0.044
		$CoCl_2 \cdot 6H_2O$	0.041
		$NaMoO_4 \cdot 2H_2O$	0.025
		$NiCl_2 \cdot 6H_2O$	0.020

(1) The same nutrient recipe except for the concentration of KNO_3 and $(NH_4)_2HPO_4$ was used for nutrient recirculation to the lab-scale biofilter study.

After two additions of the VOC mixture (50 μL of VOC (neat) mixture per liter of nutrient medium, i.e., 25.1 μL methyl n-propyl ketone, 4.5 μL n-butyl acetate, 7.2 μL ethyl 3-ethoxy propionate, 6.75 μL toluene, 6.45 μL p-xylene) had been degraded, the culture was incubated in a 20 L carboy containing the same nutrient medium used in the bottles. When the culture was able to degrade four repeated injections of the VOC mixture within about two weeks, the polyurethane packing foam was dipped into the culture solution and compressed to remove excess moisture. In this way, the moisture

content of the foam was adjusted to approximately 65% (wet basis) prior to placement in the biofilter column. As observed in the previous study conducted by Kazenski (2000), no strong substrate interactions were observed while growing the inoculating culture in the batch liquid culture.

3.1.5 Bioreactor Operation

Biofilter Experiment I

In the first set of biofilter experiments, the experimental bioreactor (Figure 3-1) was supplied with a five component paint VOC mixture (see Table 3-2) for the first 20 days of operation at a total VOC loading rate of 32 g/m^3-hr and a 1 minute residence time. Following this start up period, the biofilter was supplied toluene only for the next 75 days (see Table 3-4) in order to optimize the operation of the biofilter and to simplify the variables that can affect the performance of the biofilter.

Table 3-4: Operating conditions for the first 90 days of the polyurethane foam bioreactor experiments.

Operating Period	Pollutant Concentration (ppm_v)	Residence Time (min)	Nitrogen Supply Rate (g N/day as NO_3)
I (Day 0 – 20)	methyl propyl ketone: ~104 ppm_v, toluene: ~23 ppm_v, n-butyl acetate: ~17 ppm_v, p-xylene:~20 ppm_v , ethyl-3 ethoxy-propionate: ~32 ppm_v, Total: ~196 ppm_v	1 min	0.16 g N/ day as NO_3
II (Day 21 – 40)	Toluene: ~190 ppm_v	1 min	0.93 g N/ day as NO_3
III (Day 41 – 46)	Toluene: ~50 ppm_v	2 min	0.40 g N/ day as NO_3
IV (Day 47 – 58)	Toluene: ~110 ppm_v	2 min	0.80 g N/ day as NO_3
V (Day 59 – 68)	Toluene: ~250 ppm_v	2 min	5.58 g N/ day as NO_3
VI (Day 69 – 95)	Toluene: ~200 ppm_v	1 min	1.86 g N/ day as NO_3, & 5.58 g N/ day as NO_3 (during Day 76 to 88)
VII (Day 96 – 107)	methyl propyl ketone: ~160 ppm_v, toluene:~40 ppm_v	1 min	1.86 g N/ day as NO_3
VIII (Day 108 – 128)	methyl propyl ketone: ~104 ppm_v, toluene: ~23 ppm_v, n-butyl acetate: ~17 ppm_v, p-xylene:~20 ppm_v , ethyl-3 ethoxy-propionate: ~32 ppm_v, Total: ~196 ppm_v	1 min	1.86 g N/ day as NO_3

As can be seen in Table 3-4, toluene feed experiments were conducted with empty bed contact times (EBCTs) ranging from 1 to 2 minutes and influent toluene concentrations ranging from 50 ppm_v to 250 ppm_v. After the biofilter reached steady removal of toluene, the operating conditions of the biofilter were set to a 1 minute EBCT and a nutrient supplying rate of 1.86 g N/ day as NO_3. The biofilter was then operated with a mixture of methyl propyl ketone (MPK) and toluene for the next 12 days (Period VI). Finally, during the rest of the biofilter operational period (from Day 108 to Day 128, Period VII),

the biofilter was provided the surrogate paint VOC mixture (Table 3-2) at the same loading rate as used at the beginning of the biofilter experiments (i.e., 32 g/m^3-hr).

Biofilter Experiment II

Based on the results of the first set of experiments, it was hypothesized that the order in which the VOCs were introduced to the biofilter column may affect the VOC degradation capacity of the biofilter. To test this hypothesis, two biofilters of identical design (see Figure 3-1) were set up. The bioreactors were operated identically except that in one of the biofilters (the Continuously Fed Column, CFC), the full five-component surrogate paint mixture was supplied continuously to the bioreactor from the beginning of operation. In the second biofilter (the Sequentially Fed Column), the paint VOCs were supplied to the biofilter in a sequential manner as noted in Table 3-5. To minimize the possibility of nitrogen limitations, a concentrated nitrogen solution was added to the biofilter every day by spraying a nutrient solution over the top of the column for 10 to 20 minutes (See Table 3-6).

Table 3-5: Operating conditions during the second set of baseline biofilter experiments.

Parameters	Continuously Fed Column (CFC)	Sequentially Fed Column (SFC)
Packed bed volume	14.2 L	14.2 L
Gas flowrate	17.3 L/min	17.3 L/min
Empty Bed Contact Time (EBCT)	50 sec	50 sec
Inlet Loading (g-C/m^3-hr)	~ 40	~ 40
VOC composition	VOC mixture: methyl propyl ketone(97ppm_v), Toluene(17ppm_v), n-butyl acetate(20ppm_v), p-Xylene(17ppm_v), ethyl-3-ethoxy-propionate(31ppm_v)	1. Day 0 to Day 28: methyl propyl ketone(100 ppm_v), Toluene (100 ppm_v) 2. Day 29 to Day 52: VOC mixture

Table 3-6: Nutrient supply during the second set of biofilter experiments.

Period	Day	Concentration		Nutrient Spraying Frequency
		Nitrate	Ammonia	
I	1 – 14	10.1 g/L KNO_3	1.32 g/L $(NH_4)_2HPO_4$	10 min once per day
II	15-24	20.2 g/L KNO_3	1.32 g/L $(NH_4)_2HPO_4$	10 min once per day
III	25-52	20.2 g/L KNO_3	1.32 g/L $(NH_4)_2HPO_4$	20 min once per day

* On Day 23, the nutrient solution was not sprayed.

3.2 RESULTS AND DISCUSSION

The two sets of biofilter experiments (biofilter experiment I and II) were conducted to investigate the biodegradation pattern of surrogate paint VOC mixtures in a

biofilter and to determine the factors which control overall VOC removal. The results are presented in following two sections.

3.2.1 Biofilter Experiment I

The first set of biofilter experiments was conducted to investigate the parameters that affect VOC removal in biofilters treating surrogate paint VOC mixtures. The results of these experiments are presented below for each VOC feed experiment (bioreactor start-up/toluene feed experiments, MPK/toluene feed experiments, and paint mixture feed experiments).

Bioreactor Start Up and Toluene Feed Experiments (Period I – Period VI)

Figure 3-2 presents the inlet loading rate (i.e., mass VOC carbon supplied per unit volume of packing per unit time) and pollutant elimination capacity, EC (i.e., mass VOC carbon degraded per unit volume of packing per unit time) for the first 95 days of operation.

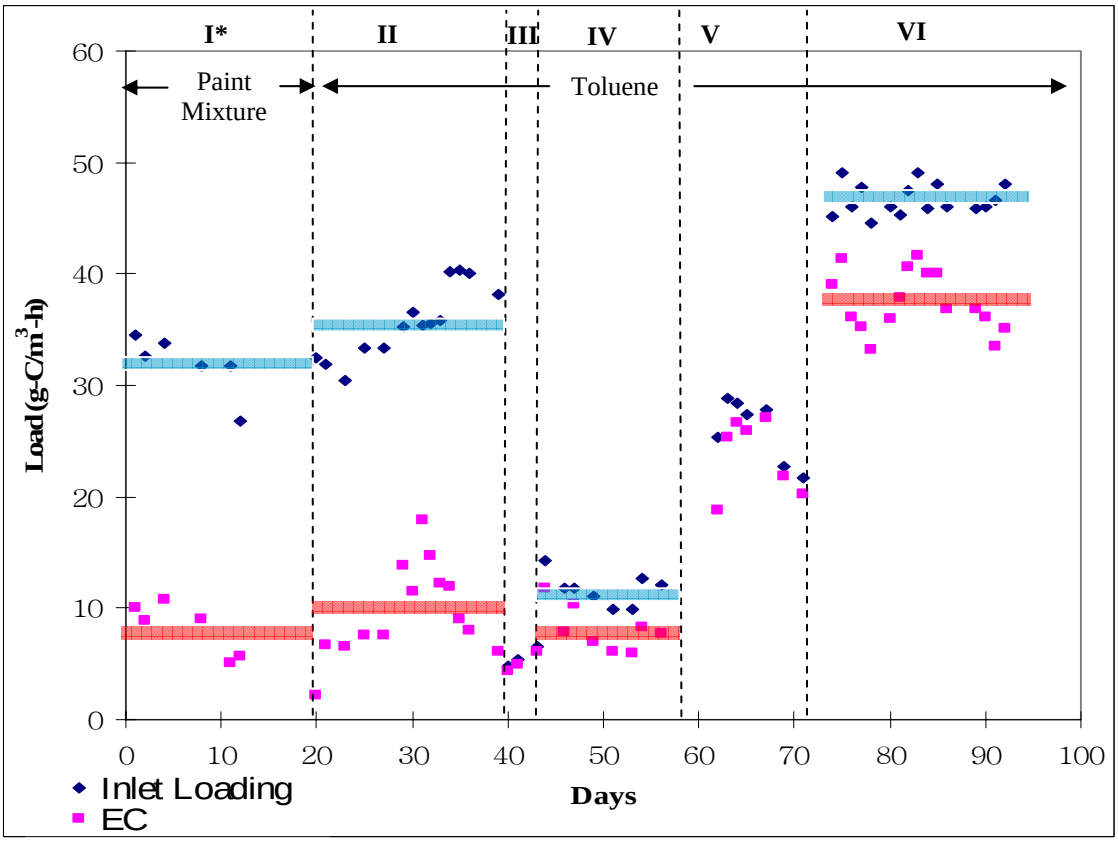


Figure 3-2: Pollutant elimination capacity and removal efficiency in the bioreactor packed with polyurethane foam packing during the first 95 days of operation. (* Table 3-3 provides detailed operating conditions and nutrient supply rates for each operational period (I-VI))

During the 20-day start-up period (Period I) when the biofilter was supplied a five-component paint VOC mixture, less than 30% overall VOC removal was achieved. No removal of toluene, xylene, or methyl propyl ketone (MPK) was observed as evident in the VOC removal profile presented in Figure 3-3. Despite the fact that the inoculation culture was capable of degrading all five of the surrogate paint VOCs in batch tests, only ethyl 3-ethoxypropionate (EEP) and n-butyl acetate (NBA) were degraded in

the biofilter. Interestingly, even MPK which is a soluble and easily degradable compound was not degraded. One possible explanation for the poor removal of MPK and the aromatic hydrocarbons in the biofilter was that the nitrogen supply to the biofilter may have been inadequate during the start up period.

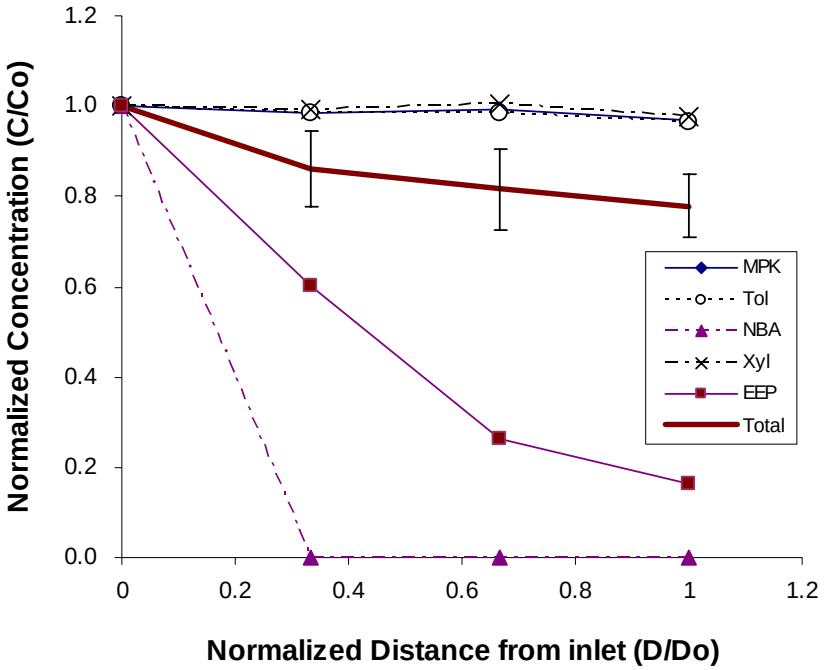


Figure 3-3: Average VOC removal profile across the column during the start up period (From Day 0 to Day 19).

To simplify the biofilter system and delineate the operating parameters that were controlling VOC removal in the system, the paint mixture feed to the biofilter was replaced with a toluene only feed for the next 75 days of operation. To investigate the possibility that the poor VOC removals initially observed may have been due to an inadequate nitrogen supply, 0.16 g N/day, the nitrogen supply to the bioreactor was

varied as delineated in Table 3-3. The results indicate that when the nutrient solution was supplied to the system on a daily basis, toluene removal efficiencies as high as 95% were achieved at an EBCT of 2 min and inlet concentration of 250 ppm_v (Period V). When the EBCT was reduced to 1 min, toluene removal efficiencies decreased to approximately 80% (Period VI). Toluene elimination capacities in excess of 40 g-C/m^3-h were achieved when there was daily addition of nutrients. Supplying nutrients less frequently (3 day or 7 day intervals) always led to a reduction in the contaminant removal efficiency across the biofilter. Daily nitrogen addition corresponded to a nitrogen supply of 5.6 g N/day to the bioreactor column which is almost a factor of 5 higher than that supplied during the first 20 days of bioreactor operation with the paint mixture. These results suggest that nitrogen limitation contributed to the poor performance of the column during start up. However, once the nitrogen supply was adequate, steady removal of the toluene was observed.

MPK and Toluene Feeding (Period VII)

When the biofilter achieved steady removal of toluene, the biofilter was exposed to a mixture of MPK and toluene (Period VII). The biofilter responded well with respect to overall VOC removals as evident in Figures 3-4 and 3-5.

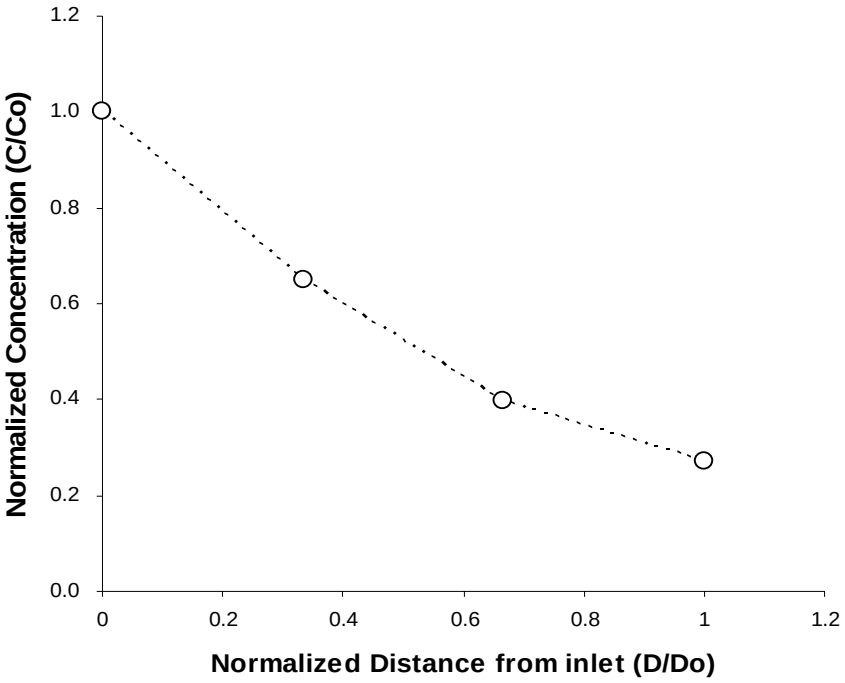


Figure 3-4: Toluene removal profile on Day 92 of operation (toluene-only feed, EBCT: 1 min, inlet toluene concentration: 200 ppmv)

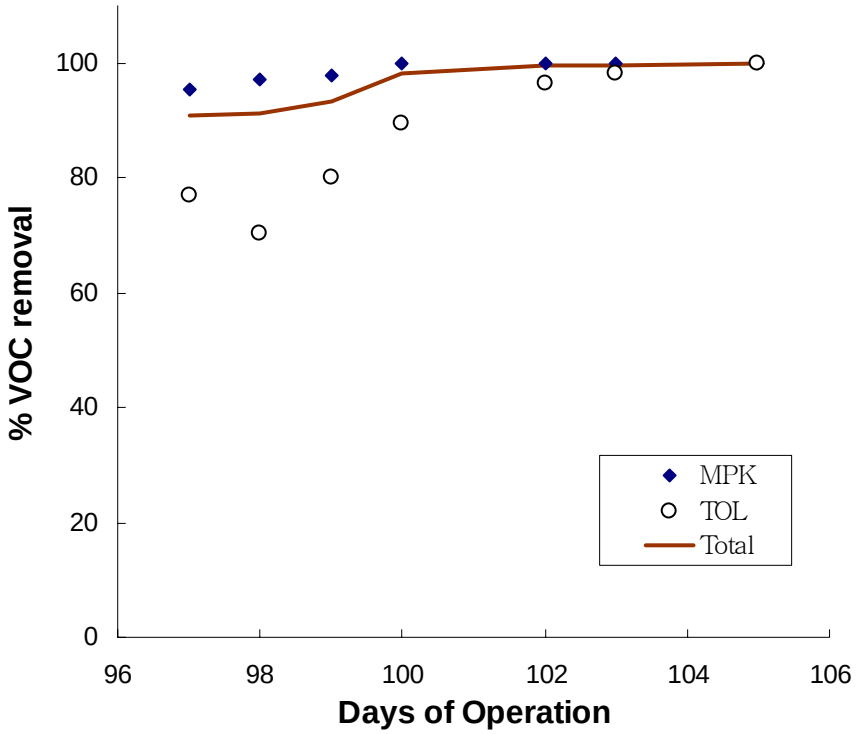


Figure 3-5: VOC removal efficiencies from Day 97 to Day 105 during the MPK/toluene mixture feeding experiments

Figure 3-4 shows the toluene removal profile across the biofilter column prior to the switch from a toluene-only feed to the MPK/toluene mixture. Upon switching feeds, greater than 90% overall removal was achieved immediately. The removal efficiency gradually increased to greater than 99% within 9 days corresponding to an elimination capacity of approximately 30 g-C/m^3-h for the mixture of MPK and toluene (Figure 3-5). The toluene removal efficiency was 75% one day after the switch but gradually increased to greater than 99% when the MPK/toluene mixture was provided (Figure 3-6 and 3-7).

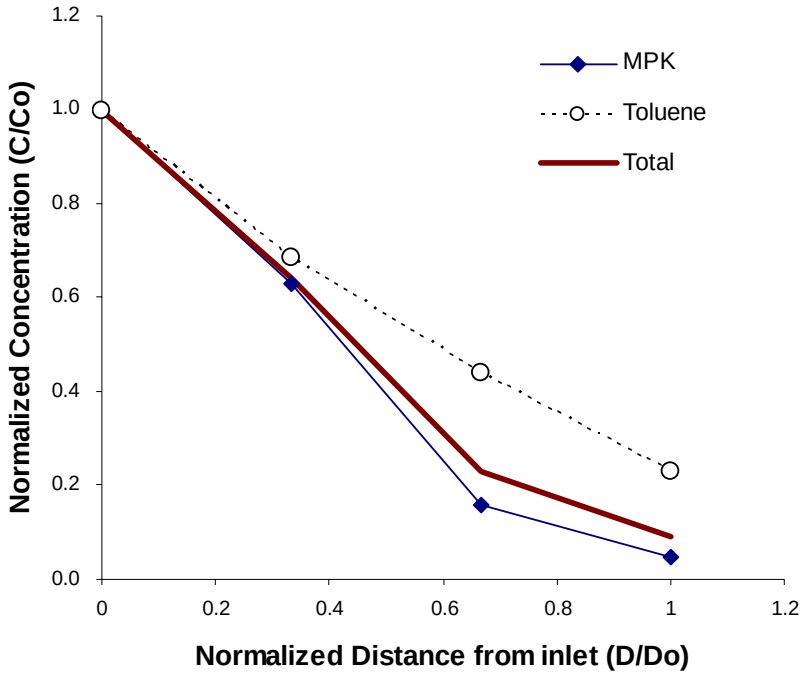


Figure 3-6: Methyl Propyl Ketone (MPK) and toluene removal profiles on Day 97 (one day after switching from a toluene-only feed to a MPK/toluene mixture feed).

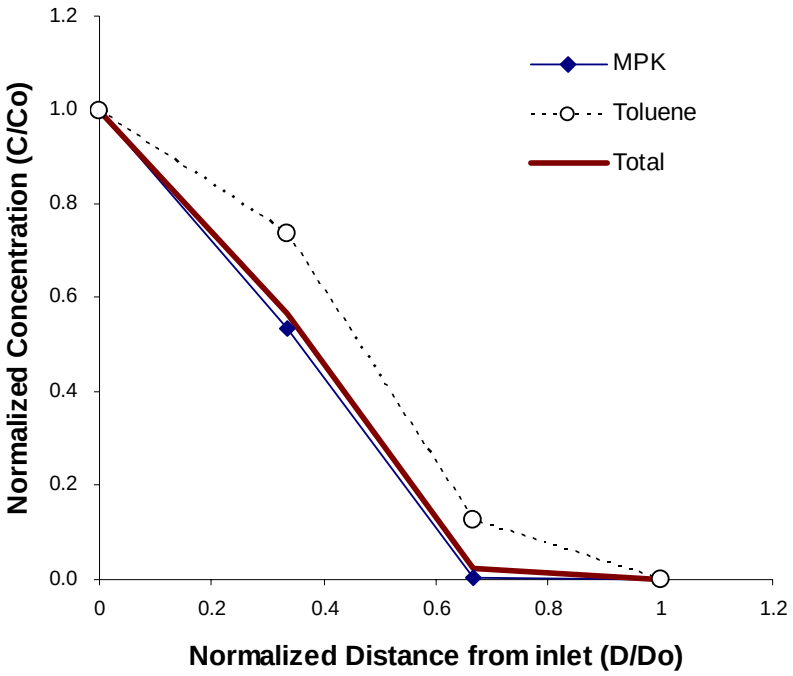


Figure 3-7: Methyl Propyl Ketone (MPK) and toluene removal profiles on Day 105 (nine days after switching from a toluene-only feed to a MPK/toluene mixture feed).

Visual inspection of the packing media indicated that the addition of MPK facilitated biomass development on the packing. Ultimately, approximately 160 mg COD/g foam biomass was established on the packing during the MPK/toluene feed period. This biomass, in turn, likely aided in nutrient retention and toluene removal in the packing media.

Paint Mixture Feeding (Period VIII)

After a thick biofilm was established in the biofilter, the five-component surrogate paint mixture was again supplied to the biofilter from Day 108 to Day 128. The biofilter achieved approximately 91% overall VOC removal efficiency one day after the chemical feeding was switched from the MPK/toluene mixture to the paint mixture. With the exception of xylene, VOCs were degraded simultaneously in the column suggesting that VOC substrate interactions did not severely inhibit biodegradation (Figure 3-8, 3-9).

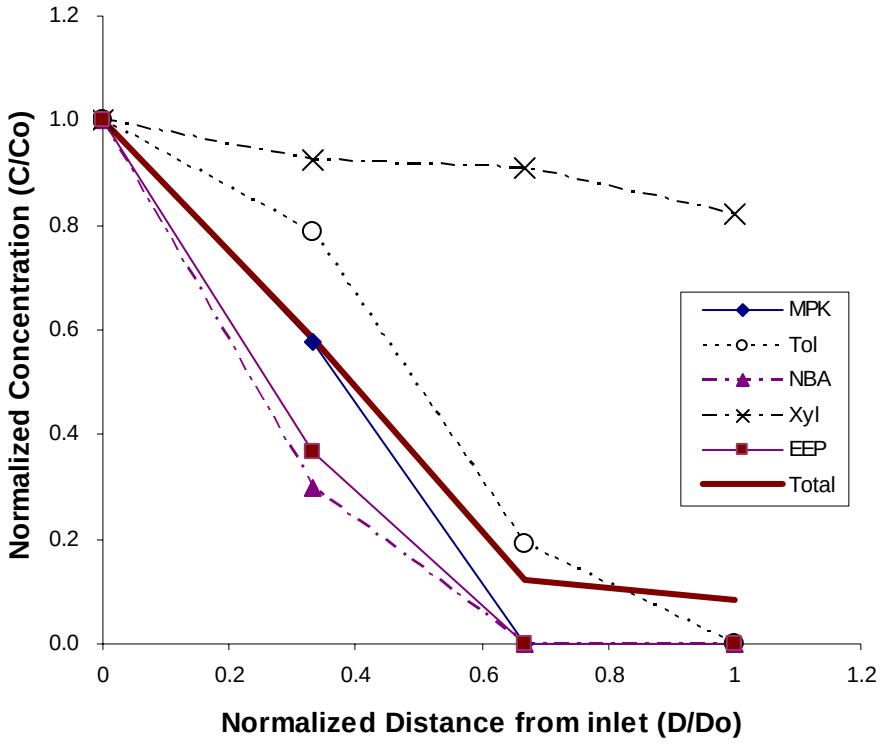


Figure 3-8: Paint mixture removal profile on Day 109 (one day after switching from the MPK/toluene mixture feed to a surrogate paint mixture feed)

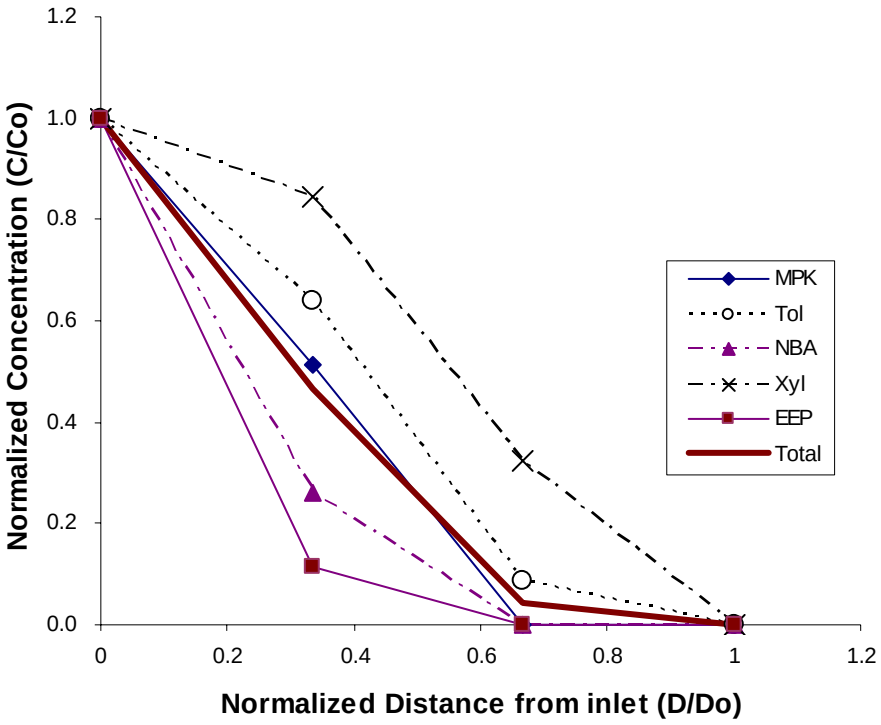


Figure 3-9: Paint mixture removal profile on day 119 (ten days after switching from a MPK/toluene mixture feed to a surrogate paint mixture feed).

Following the switch to the paint mixture, poor p-xylene removal was initially observed with less than 20% degraded over the entire bioreactor column (Figure 3-8). However, within 10 days, xylene-degrading activity was fully established and a removal efficiency of greater than 99% was observed (Figure 3-9). In a previous study of a biotrickling filter packed with polypropylene pall rings and inoculated with the same culture used on this study, the total maximum VOC removal efficiencies was 95% but the toluene and xylene removal efficiencies were only 80% and 60%, respectively even after 53 days of operation (Table 3-6)(Kazenski 2000).

Table 3-7: Comparison of operating conditions and removal efficiencies observed in the current study and in a previous study by Kazenski.

	Current study	Kazenski *et al*. (2000)
Operating Mode	Biofilter	Biotrickling filter
Packing media	Polyurethane foam	Polypropylene pall ring
Total inlet VOC Conc.(ppm_v)	182	200
Inlet loading (g C/m^3-h)	30	31
Contaminants (inlet conc.)	Methyl n-propyl ketone (93 ppm_v), toluene (23 ppmv), n-butyl acetate (17 ppmv), p-xylene (19 ppm_v), ethyl-3 ethoxy-propionate (30 ppm_v)	Methyl n-propyl ketone (110 ppm_v), toluene (20 ppmv), n-butyl acetate (20 ppmv), p-xylene (18 ppm_v), ethyl-3 ethoxy-propionate (32 ppm_v)
Empty Bed Contact Time (EBCT)	1 min	1 min
Nutrient delivery method	Nutrient spray every 3 days	Nutrient recirculation (2.7 L/min)
Removal efficiency	> 99%	95% (> 99% MPK, 80% toluene, > 99% NBA, 60% p-xylene, > 99% EEP)

In Kazenski's study, no attempt was made to enrich the aromatic-degrading microbial population within the biotrickling filter. In this study, feeding the biofilter toluene and then the MPK/toluene mixture appears to have enhanced biofilm development and aromatic hydrocarbon degradation. As toluene and xylene appear to limit overall VOC removal in biofilters treating paint mixtures, establishing the aromatic hydrocarbon degrading population first may enable higher aromatic removals for paint booth applications. Another possible explanation for the difference in performance between the biotrickling filter and the polyurethane biofilter is that the wet conditions present in biotrickling filters tend to inhibit the mass transfer of hydrophobic compounds.

However, the polyurethane foam used in the biofilter was hydrophilic and thus the moisture content of the biofilter media was as high as 75% (wet basis). Thus, the hydrophilic foam biofilter would not be expected to have much of an advantage over biotrickling filters in this regard.

3.2.2 Biofilter Experiment II

Even though high removals of paint VOCs were ultimately achieved in the previous biofilter experiments (I), it was not clearly delineated what role feeding toluene alone to the bioreactor had in improving the capacity of the biofilter to treat the paint VOC mixture. It was hypothesized that a sequential feeding strategy in which the paint VOCs are provided to the bioreactor in a sequential manner starting with the most recalcitrant compounds first may be a key factor that leads to more complete removal of the paint VOC mixture. For the paint VOC mixture, this strategy would suggest that feeding the bioreactor column toluene only initially might establish a sufficient aromatic hydrocarbon degrading microbial population to remove more of the toluene when the full paint mixture is present in the feed. Results of the first set of baseline experiments also indicate that adding MPK prior to feeding the full paint VOC mixture might also help establish the biomass on the foam.

To investigate the merits of this feed strategy, two identical biofilters were set up and operated in an identical fashion except for the composition of the chemical feed. In one reactor (i.e., the continuously fed column, CFC), the bioreactor was provided the five-component feed mixture continuously from the beginning of operation. In the second identical reactor (the sequentially fed column, SFC), the paint VOCs were provided to the bioreactor sequentially in a manner similar to those in biofilter experiment I. However, a slightly modified sequential feeding strategy was introduced in this experiment.

Instead of feeding toluene only at the beginning of the experiment, a mixture of toluene and MPK was initially supplied to the SFC instead of just toluene. It was hypothesized that the addition of MPK in the SFC might facilitate biomass establishment which would improve nitrogen retention within the biofilter. As noted in the baseline biofilter experiment I described earlier, the higher nitrogen availability led to greater VOC removals.

Figure 3.10 shows the overall VOC removal observed in the continuously and sequentially fed columns. As observed in the previous experiment, the overall VOC removal in the column which was supplied the paint VOC mixture continuously (the Continuously Fed Column) was low. Only NBA and EEP were degraded and the other three chemicals were not removed even following 29 days of operation. However, in the other column which was initially supplied with a toluene/MPK mixture (the Sequentially Fed Column), more than 80% overall VOC removal was achieved (see Figures 3-11 and 3-12).

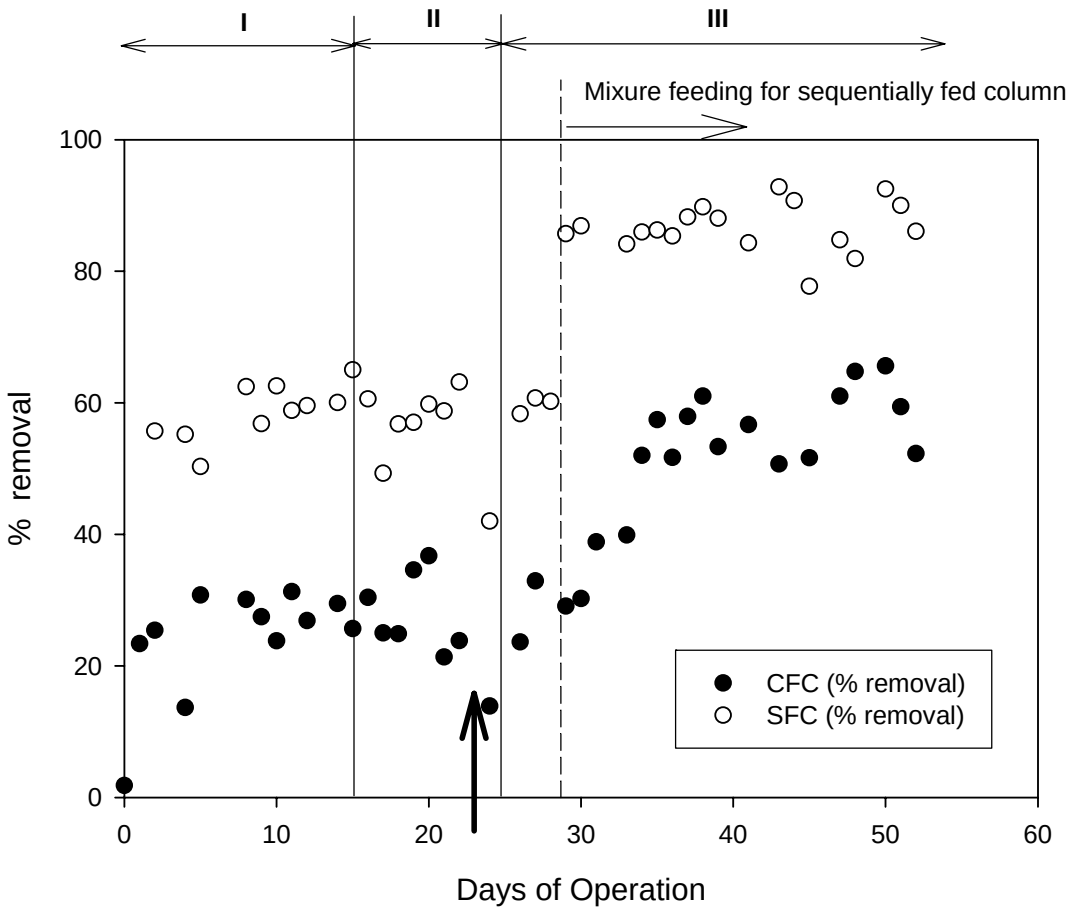


Figure 3-10: Overall VOC removal in the continuously and sequentially fed columns. (Note: The arrow indicates when the nutrient supply was discontinued for one day and the number (I, II and III) at the top of the figure corresponds to the operating conditions summarized in Table 3-5)

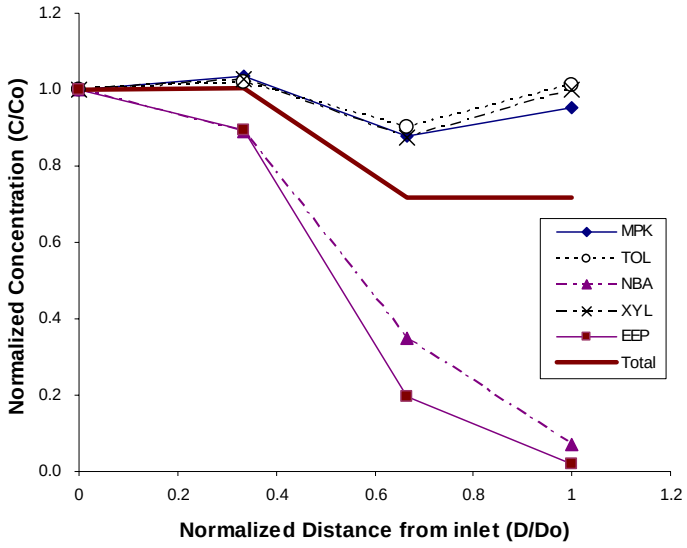


Figure 3-11: Paint mixture removal profile on Day 29 in the Continuously Fed Column (CFC)

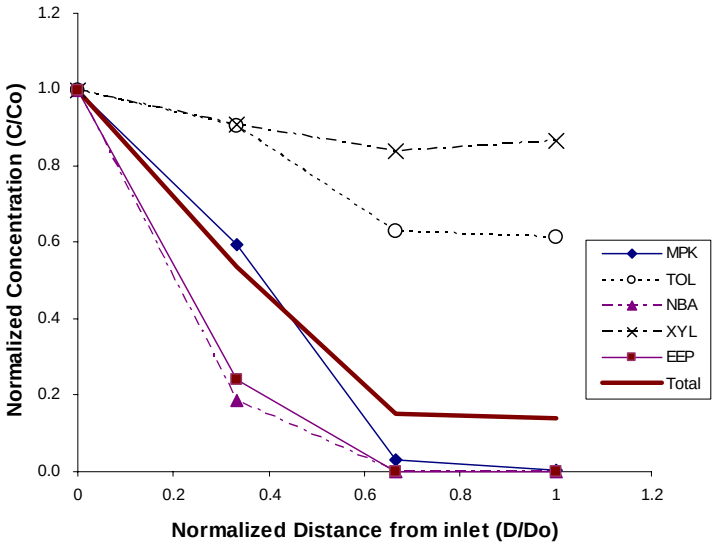


Figure 3-12: Paint mixture removal profile on Day 29 in the Sequentially Fed Column (SFC) (one day after switching from the methyl propyl ketone (MPK)/Toluene feeding to a surrogate paint mixture).

Within the next two weeks of biofilter operation, MPK removal in the continuously fed column slowly increased to 40%, but no toluene or p-xylene was removed. However, in the sequentially fed column, toluene removal gradually increased to approximately 70% and by Day 43, 50% of the xylene was also removed. Figure 3-13 depicts the elimination capacity (EC) of these two biofilters measured at the end of experiments on Day 52. The EC in the sequentially fed column was two times greater than the EC in the continuously fed column. One possible reason for the higher EC in the sequentially-fed column can be explained by comparing the biomass quantity and available nitrogen concentration on the foam (see Figures 3-14 and 3-15).

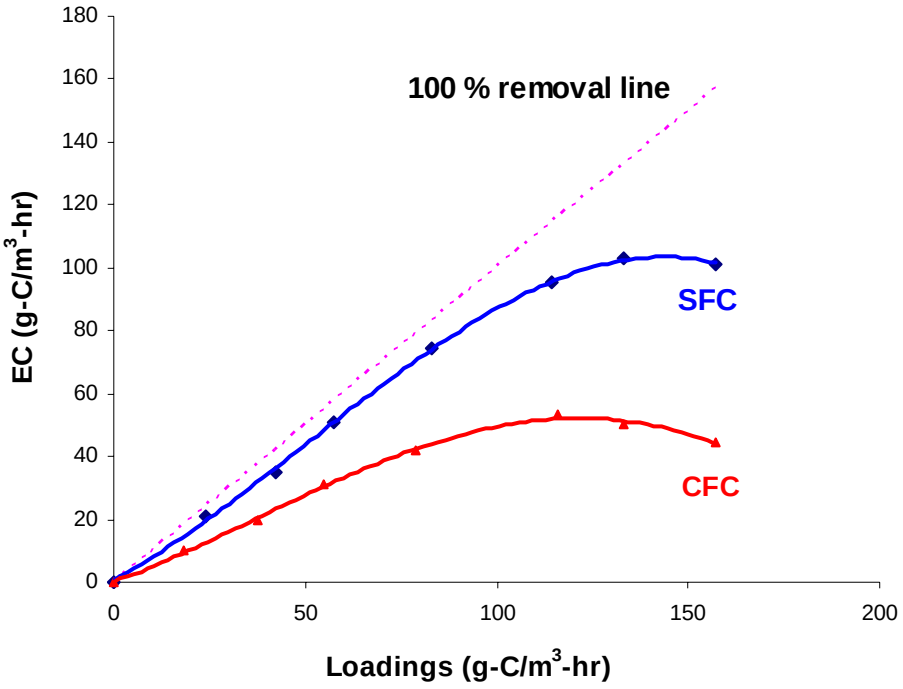


Figure 3-13: VOC Elimination Capacity (EC) in the continuously fed (CFC) and sequentially fed (SFC) biofilters.

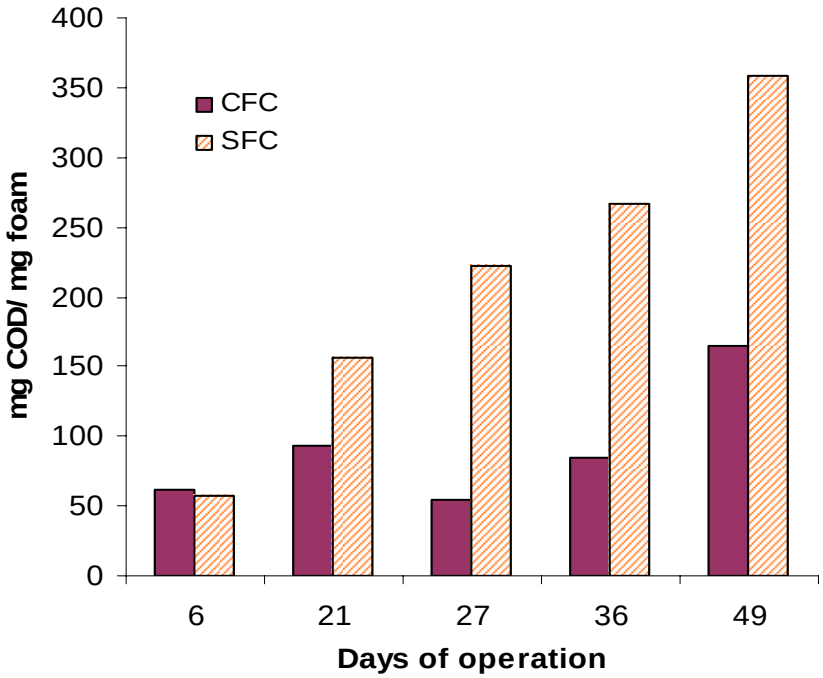


Figure 3-14: Biomass quantity (COD) in the SFC and CFC biofilters as a function of operating period.

As can be seen in Figure 3-14, the quantity of biomass established on the foam in both biofilters was similar on Day 6. However, a much greater quantity of biomass (approximately 2 times higher) accumulated in the sequentially fed column over time.

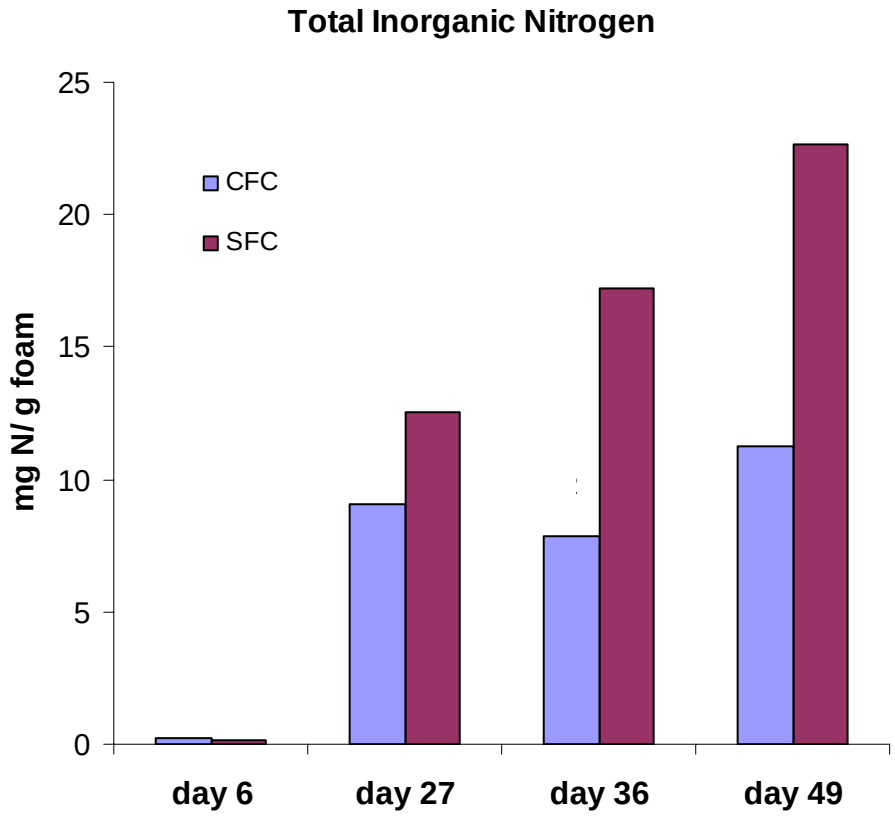


Figure 3-15: Comparison of total inorganic nitrogen available (NO_3^--N + NH_4^+-N) in the bioreactor packing material.

Even though the biofilters were identical and inoculated with the same culture (and provided the same quantity of nitrogen), the available nitrogen on the foam was much different in both columns (see Figure 3-15). This difference seems to be a result of the different feeding strategies employed in each reactor. The relatively easily degradable compound, MPK, facilitated biomass growth in the sequential feeding column. This, in turn, yielded increased biomass which enhanced nutrient retention. Thus, the sequential feeding column was able to achieve higher ECs, greater biomass accumulation and nitrogen retention. In contrast, the MPK degradation was inhibited during the start-up period in the continuous feeding column. As a result, less biomass

accumulated in the CFC system which likely hindered nitrogen retention in the system and further biomass accumulation.

Although the SFC achieved higher overall VOC removal than the CFC in this experiment, the aromatic removal was not as high as that observed in the previous biofilter experiment (Experiment I) described in Section 3.2.1. The reason for this difference is not known. However, these results suggest that either feeding MPK and toluene together inhibited the toluene -degrading microbial population or the microbial population present in the SFC preferred the MPK substrate over toluene. One common result derived from both baseline biofilter experiments was that a microbial population capable of degrading aromatic hydrocarbons should be enriched in the bioreactor to ensure high removal of the aromatic compounds.

3.4 SUMMARY

During the first set of baseline experiments, very poor removal of the paint VOC mixture was observed. When the feed mixture was replaced with a single component, toluene, high removals were only achieved after sufficient nitrogen was provided indicating that nitrogen limitations played a role in the initial poor performance of the bioreactor. The VOCs were then reintroduced to the bioreactor in a sequential manner (i.e., first, toluene only, then an MPK/toluene mixture and, finally, a mixture of toluene, xylene, MPK, EEP and NBA) and overall removals of greater than 99% were ultimately achieved for the paint mixture. In the second set of baseline experiments, the merits of a sequential feed strategy were investigated further by comparing the performance of a sequentially fed column to that of a continuously fed column. The sequentially fed column accumulated biomass (and nitrogen) more rapidly and achieved higher VOC

elimination capacities than the continuously fed column. These results indicate that acclimation history (i.e., the order in which a biofilter is acclimated to each VOC) can affect the ultimate VOC degradation capacity of the system.

It was unclear from these experiments, however, what role the microbial inoculum played in the VOC degradation patterns observed during the baseline experiments. The same inoculum was used in each of the baseline experiments and it is unclear how the development of this inoculum may have affected the subsequent performance of the bioreactors. Similarly, the effect of different VOC feed strategies and nitrogen availability on the actual composition of the microbial population within the biofilter was not delineated. Monitoring the microbial population as a function of acclimation history and nutrient condition over extended operation may provide additional insights into the relationship between the composition of the microbial population in the biofilter and substrate utilization within a biofilter treating VOC mixtures.

Chapter 4 Effect of Acclimation Method on the Degradation of Paint VOC Mixtures

In biological treatment systems, pollutant degradation patterns will be a function of the pollutant mixture fed to the bioreactor as well as the microbial population present in the reactor. The microbial community present in the reactor is expected to be a function of the inoculation method employed, the acclimation history of the biological system and the period of operation. Mixed microbial cultures are the norm for biofilters and other biological treatment systems placed in the field since maintaining a pure culture is not feasible, and in many cases, not desirable. The composition of this mixed culture (and its pollutant degradation capacity) may depend on the method used to develop and transfer the inoculum to the bioreactor. Even in systems which start with the same microbial culture, previous research studies have shown that the degradation of the pollutants in the system can be a function of how the system was acclimated to the pollutants (Kar 1996; Swaminathan 1999).

In the previous experiments described in Chapter 3, severe substrate interactions were observed during the start-up period when the biofilter was supplied a five-component surrogate paint mixture. Degradation of the aromatic compounds in particular was severely repressed during the start-up period. Biofilter performance improved significantly after the nitrogen supply to the bioreactor was increased and the biofilter was acclimated sequentially to the paint VOC mixture. Subsequent experiments indicated that a bioreactor that was acclimated to the paint VOCs in a sequential manner (starting with toluene) achieved higher paint VOC removals than did a bioreactor that was supplied the paint mixture continuously. These results suggest that enriching the microbial population capable of degrading aromatic hydrocarbons via a sequential feed

strategy is beneficial for achieving high removals of these compounds. However, it was unclear from these experiments whether simply enriching the culture used to inoculate the bioreactor would have been sufficient to improve performance or if a sequential VOC feed strategy after biofilter startup is necessary, To address these questions, another series of experiments were conducted to compare the performance of the SFC and CFC. In these experiments, however, the culture used to inoculate the bioreactors was developed in a manner to preserve as much of the diversity of the VOC-degrading microbial population as possible. The effect of acclimation history (e.g., sequential versus continuous VOC feed) on VOC degradation patterns was then assessed as described below. The effect of acclimation history on the composition of the microbial population was also delineated using DGGE as discussed in the next Chapter.

4.1 EXPERIMENTAL METHODS

During this experiment, two identical biofilters (Figure 3-1) were set up and operated for a total of 269 days. One of these biofilters was operated for a period of 122 days as a sequentially fed column while the second biofilter was supplied the surrogate paint VOC mixture continuously from start up (i.e., a continuously fed column). The method used to develop the microbial inoculum for the columns as well as the operation of the bioreactors are described in the sections below. The same analytical methods described earlier in Chapter 3 (Section 3.1) were utilized to measure the VOC degradation, nitrogen content and biomass accumulation in each biofilter.

4.1.1 Inoculation

During the baseline experiments reported in Chapter 3, the inoculating culture was grown up in a single liquid batch culture in the presence of all five of the VOC compounds that comprised the surrogate paint mixture. The ratio of each VOC provided to the cultures was proportional to its expected fraction in the paint waste gas stream and, as a result, toluene and xylene comprised only approximately 20% of the total VOCs provided. Although the intent was to select for organisms capable of degrading all the components of the VOC mixture, successive incubations may have diminished the microbial population capable of degrading aromatic hydrocarbons. This may have contributed to the poor start up results observed in the previous baseline experiments and may explain the success of the sequential feed approach. To investigate this possibility and ensure that the bioreactor was inoculated with the most diverse culture possible, the initial inoculum for the biofilter experiments described in this chapter was obtained from wastewater treatment sludge. Five separate inoculum solutions were grown up in 250-mL amber bottles in the presence of 100 mg-C/L of one of the five VOCs (Table 3-1) as the sole carbon and energy source. When each individual culture was able to degrade three injections of a single VOC, the five cultures were mixed together in a carboy containing 5L of the same nutrient medium used in the bottles (see Table 3-4). This mixed inoculum was then fed a mixture of five paint VOCs (100 mg –C/L of each VOC). Once this VOC mixture had been degraded, the inoculating culture was recirculated through the bioreactor packing material for 12 hrs prior to start up. It was hypothesized that the development of separate microbial cultures for the biofilter inoculum would improve the degradation of the aromatic hydrocarbons in the continuously fed bioreactor, particularly during the start-up period.

4.1.2 Biofilter Operation

During this experiment, two identical biofilters (Figure 3-1) were set up and operated in an identical fashion except for the composition of the VOC-contaminated waste gas provided to each biofilter. One biofilter (the Continuously Fed Column, CFC) was provided a five-component paint mixture continuously throughout the experiment while the second bioreactor (Sequentially Fed Column, SFC) was provided the VOCs in a sequential manner (i.e., first toluene, then a toluene/methyl propyl ketone mixture and finally the five-component paint mixture used in the previous baseline study) (see Table 4-1). Both biofilter columns were operated at an empty bed contact time (EBCT) of 1 min. The total VOC-carbon loading to each biofilter was maintained at approximately 33 g-C/m^3-hr throughout the experiment even though the VOC composition of the waste gas varied in the sequential fed column as a function of operating period.

Table 4-1: VOC feed composition during the CFC and SFC biofilter experiments.

Experiment	Days	VOC feed composition	
		Sequentially Fed Column (SFC)	Continuously Fed Column (CFC)
Start-up period	1-63	toluene (160 ppm_v)	paint VOC mixture
MPK/Toluene feed experiment	64-71	methyl n-propyl ketone (164 ppm_v), toluene (38 ppm_v)	
Paint mixture experiment	72-122	paint VOC mixture	

Note: Paint VOC mixture; methyl n-propyl ketone (110 ppm_v), Toluene (20 ppm_v), n-butyl acetate (32 ppm_v), xylene (20 ppm_v), ethyl-3-ethoxy- propionate (18 ppm_v)

During the column start up period (Day 0 to Day 63), the spraying frequency and composition of the nutrient medium was altered several times to optimize the performance of the biofilters (see Table 4-2). Then, during the rest of the experimental period, the nutrient supply rate was set equal to that provided in Period V in Table 4-2.

Table 4-2: Experimental conditions for each period during start-up.

Period	Day	Concentration		Nutrient Spraying Frequency
		Nitrate	Ammonia	
I	1 – 3	No	No	No
II	4 – 7	20.2 g/L KNO_3	1.32 g/L $(NH_4)_2HPO_4$	5 min once per day
III	8 – 14	20.2 g/L KNO_3	3.96 g/L $(NH_4)_2SO_4$	5 min once per day
IV	16 – 27	20.2 g/L KNO_3	3.96 g/L $(NH_4)_2SO_4$	30 min twice per day
V	28 – 44	20.2 g/L KNO_3	1.32 g/L $(NH_4)_2HPO_4$	30 min twice per day
VI	45 - 63	20.2 g/L KNO_3	3.96 g/L $(NH_4)_2HPO_4$	30 min twice per day

Note: See Table 3-3 for the rest of nutrient composition (except for the nitrogen source). On Day 15, the nutrient solution was sprayed for 15 minutes.

4.2 RESULTS AND DISCUSSION

4.2.1 Start-up Period (Day 0 to Day 63): Sequentially and Continuously Fed Columns

During the 63- day start up period, the sequentially fed column (SFC) was provided a toluene-only waste gas feed while the continuously fed column (CFC) was supplied a waste gas contaminated with five-component surrogate paint mixture. After recirculating the inoculation solution through the column overnight, no nutrients were sprayed over the top of the column to prevent the shear force of the nutrient spray from washing the biomass out of the column. It was expected that the inoculum solution itself had sufficient nutrients for the first couple of days of operation. However, within the first 3 days of biofilter operation, VOC removal in both columns dropped suggesting that

nutrient limitation was a problem. The overall VOC removal efficiency in both the SFC and CFC biofilter during the start-up period is shown in Figure 4-1.

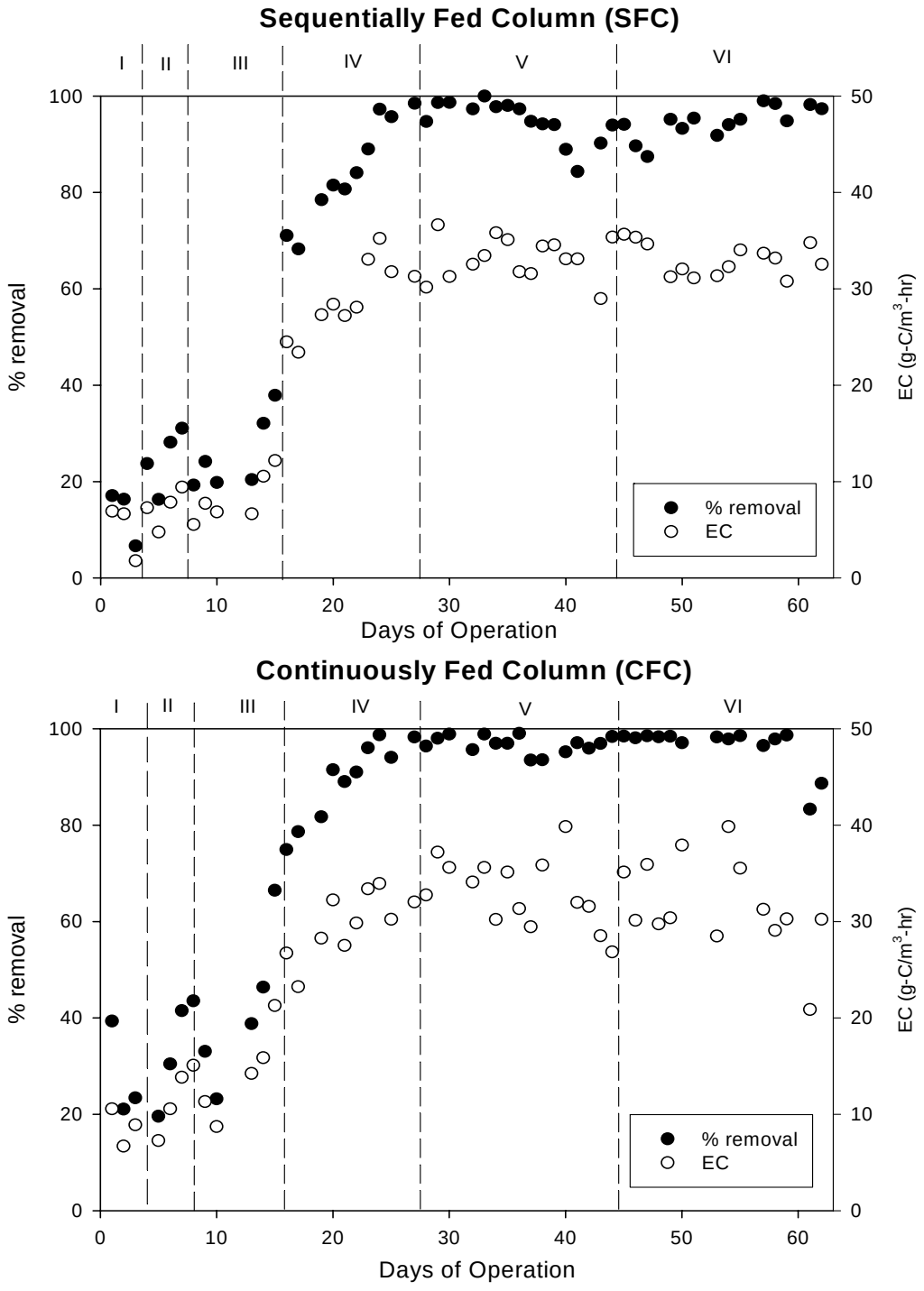


Figure 4-1: VOC removal during the start-up period (from Day 0 to 63) in the sequentially and continuously fed columns.

Following three days of nutrient-limited conditions, additional nutrients were sprayed daily over the top of the column for 5 minutes per day. Moderate improvements were observed; however, when the nutrient spraying interval was increased to 30 minutes twice per day (60 minutes per day total), a rapid increase in VOC removal was observed. Within 30 days, high VOC removals were achieved and maintained in both columns. Interestingly, the VOC removal efficiency in the CFC biofilter was as high as that achieved in the SFC during this start-up period. These results suggest that the method used to develop the inoculum for the SFC and CFC bioreactors yielded a more diverse and robust microbial culture since the cultures were individually enriched for each VOC present in the paint mixture.

Figure 4-2 presents the VOC removal profiles across the CFC biofilter during the start-up period. As was observed in previous experiments, removal of methyl propyl ketone and the aromatic hydrocarbons was poor under nutrient limited conditions, while almost complete removal of NBA and EEP was achieved in the CFC biofilter. After supplemental nutrients were provided, the MPK removal recovered most rapidly. Toluene and xylene removal efficiency improved much more slowly.

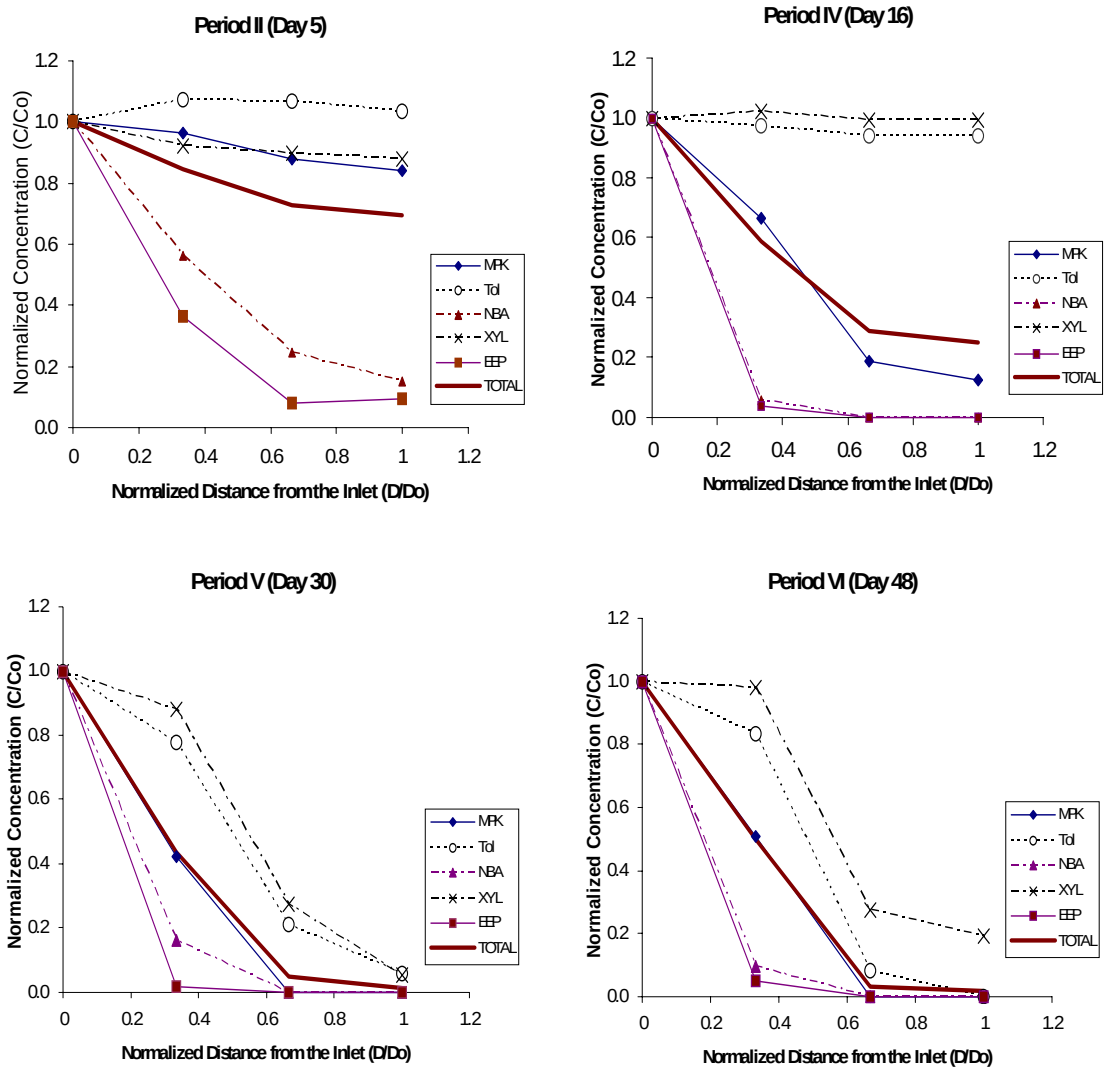


Figure 4-2: VOC removal profiles across the CFC biofilter during the start up period. (Co: VOC concentration at the inlet, C: VOC concentration at each sampling point, D: distance of each sampling point, Do: total length from inlet to outlet)

The method utilized to develop the inoculating culture for the SFC and CFC biofilter was intended to produce as diverse a VOC-degrading microbial culture as possible. However, after a mixed culture is inoculated into a column, the microorganisms

must compete with each other for the carbon substrates (i.e. VOCs) and nutrients. Microorganisms that are most competitive at the low nutrient conditions initially present in the foam biofilter likely have a competitive advantage over the organisms that require nutrient rich conditions for growth. Since n-butyl acetate (NBA) and ethyl ethoxy propionate (EEP) were readily degraded during the initial start up period when the nitrogen concentrations in the biofilm were the lowest, this suggests that the microorganisms degrading NBA and EEP were the most competitive under the nutrient-limited conditions encountered initially in the biofilters.

Even though nutrients are a crucial factor for facilitating microbial growth and improving pollutant removal in the biofilter columns, it was difficult to achieve nutrient (nitrogen) rich conditions in the biofilters during the start-up period (See Figure 4-3). Because biomass establishment on the packing medium immediately after inoculation was poor, little of the nutrient solution provided to the packing material was actually retained in the biofilm. Thus, nutrients (nitrogen in particular) may limit biomass establishment on the packing medium.

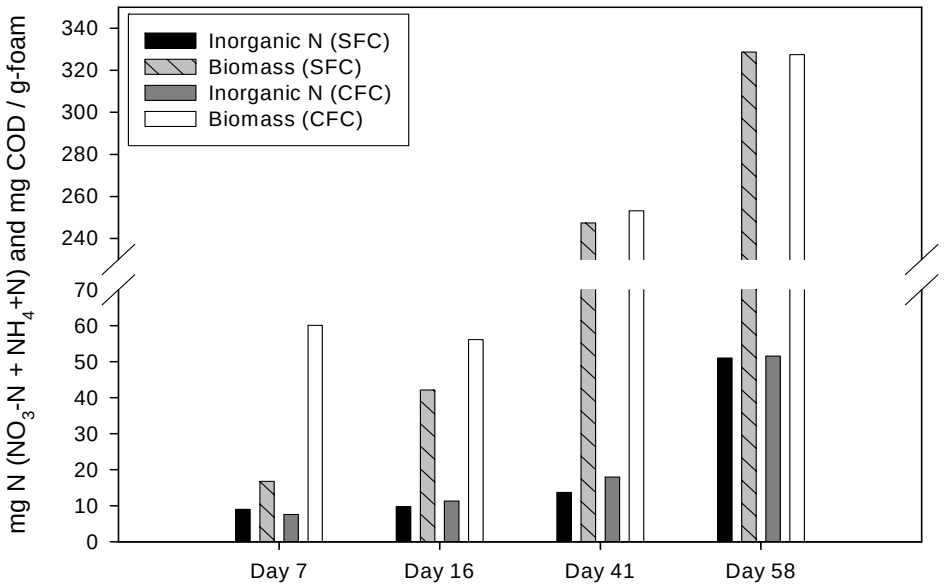


Figure 4-3: Total inorganic nitrogen concentrations and biomass quantities (COD) in the SFC and CFC biofilters during the start-up period.

Prior to Day 16 of operation, the nutrient solution was sprayed over the column only 5 minutes per day. The biomass quantity retained on the foam packing material as well as the nitrogen available in the biofilm was quite low, and as a result, the VOC removal efficiencies in both columns were low. From Day 16 onward, however, the nutrient spraying interval was increased to 30 minutes and, by Day 41, the quantity of biomass retained on the packing material was substantially greater. Similarly, the nitrogen retained within the packing material also increased; by Day 58 it was five times greater than that available during the first few weeks of operation. As nutrient availability and biomass quantity increased, the VOC removal rapidly increased. These results suggest that establishment of biomass within the column aided nutrient retention within the column and vice versa.

The poor VOC removal observed during the start-up period was likely due to a nutrient limitation caused by poor nutrient distribution and retention within the column. The VOC removal efficiency in both intermittent biotrickling filters increased significantly only after the nutrient spraying frequency was increased to 30 min, twice a day. A more concentrated nutrient solution containing 20 g/L KNO_3 and 3.96 g/L $(NH_4)_2SO_4$ did not improve performance when a short spraying interval (5 minutes) was employed. Similarly, tripling the ammonium concentration in the recirculating liquid medium had little effect on bioreactor performance at a short spraying interval. These results suggest that the nutrient distribution issue was more likely the important factor affecting VOC removal rather than the concentration of the nutrient solution itself during the start-up period.

4.2.2 MPK/Toluene feed experiment (Day 64 to Day 71): Sequentially Fed Column

After the SFC biofilter achieved steady removal of toluene, a mixture of MPK (164 ppm_v) and toluene (38 ppm_v) was supplied to the column. This pollutant loading rate is equivalent on a carbon basis to the total paint VOC mixture provided to the CFC during this period. Figure 4-4 presents the VOC removal profiles in the SFC just before and one day after switching from the toluene feed to the MPK/toluene feed. Even though the column was initially inoculated with an MPK-degrading culture, the biofilter had not been exposed to MPK for two months prior to switching the feed composition. Regardless, the recovery of MPK removal in the biofilter was quite rapid. MPK and toluene was degraded nearly simultaneously in the biofilter column suggesting that the toluene-degrading microbial culture in the biofilter was able to degrade MPK as well. A longer delay would have been expected before high MPK removal was achieved in the

biofilter if different microorganisms were responsible for the MPK and toluene degradation.

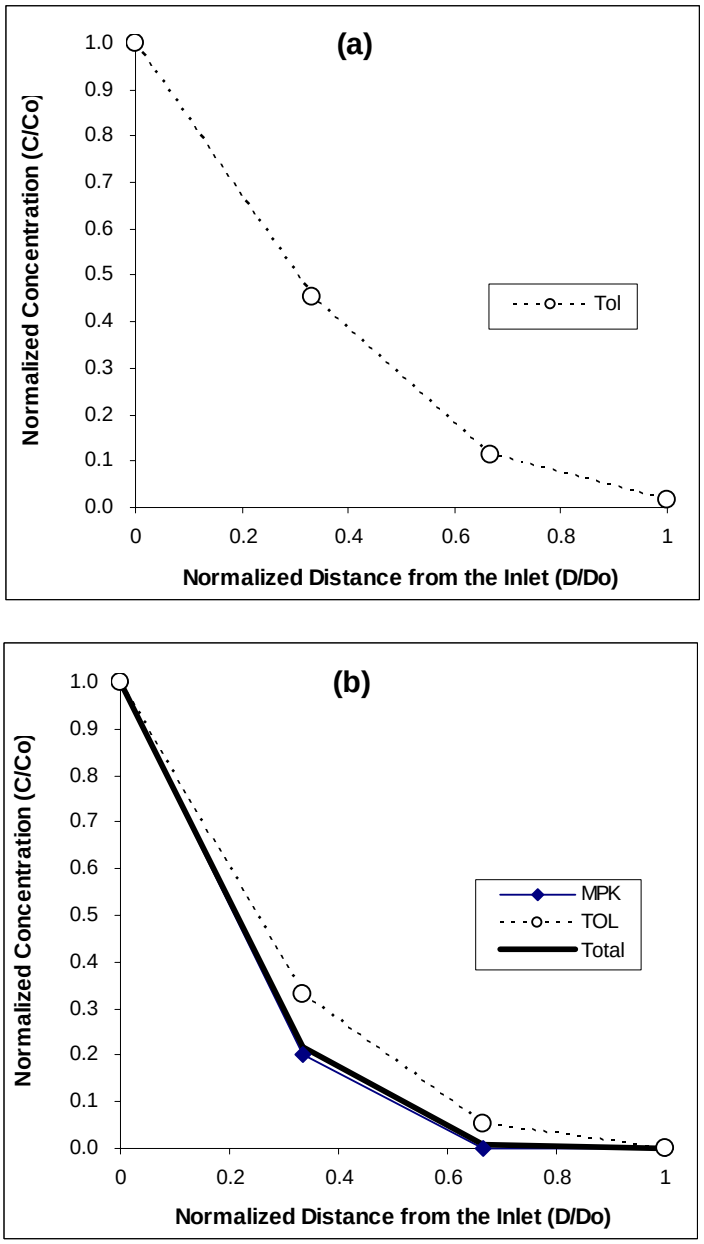


Figure 4-4: VOC removal profile across the sequentially fed column on (a) Day 58 toluene-only feed, 165 ppmv and (b) Day 64 (One day after switching from a toluene only-feed to methyl propyl ketone(163 ppmv)/toluene(38ppmv) mixture feed)

Following the switch in feed composition, removal of MPK in the column continued to improve and seven days later, most of the MPK supplied to the column was degraded in the first 25 cm of biofilter depth (see Figure 4-5).

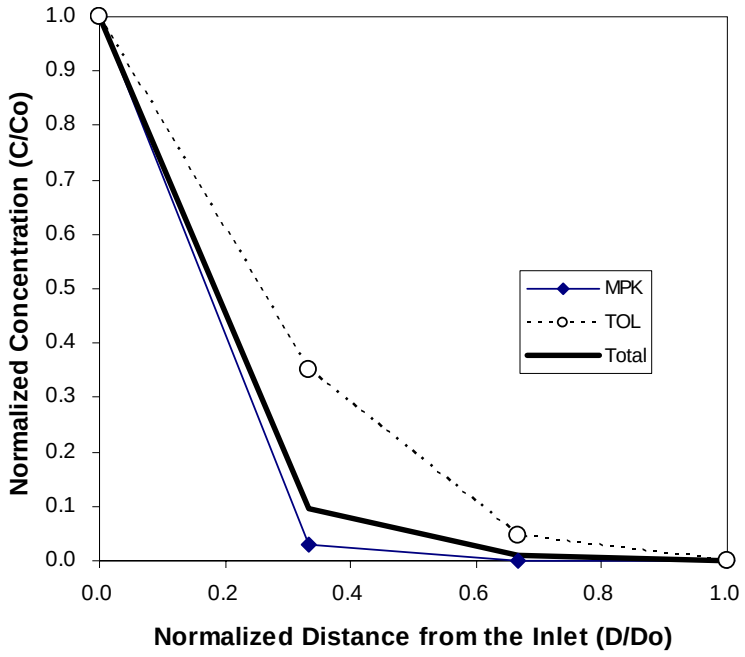


Figure 4-5: Methyl propyl ketone (MPK) and toluene removal profiles across the SFC on Day 70 (Seven days after switching from a toluene-only feed to a MPK/toluene mixture feed)

4.2.3 Paint mixture experiments (Day 72 to Day 122)

During the remainder of the experimental period, a surrogate paint mixture was supplied to the SFC. The SFC biofilter immediately removed NBA and EEP after switching from the MPK/toluene feed to the five component paint mixture. In the SFC biofilter, complete removal of MPK and 90% removal of toluene was achieved even in the presence of the other three compounds. Xylene removal efficiency was initially low (~20 %) (Figure 4-6). Ten days following the change in feed composition in the SFC,

however, xylene removal efficiency had improved to 94% and then decreased again, while toluene removal remained consistently high.

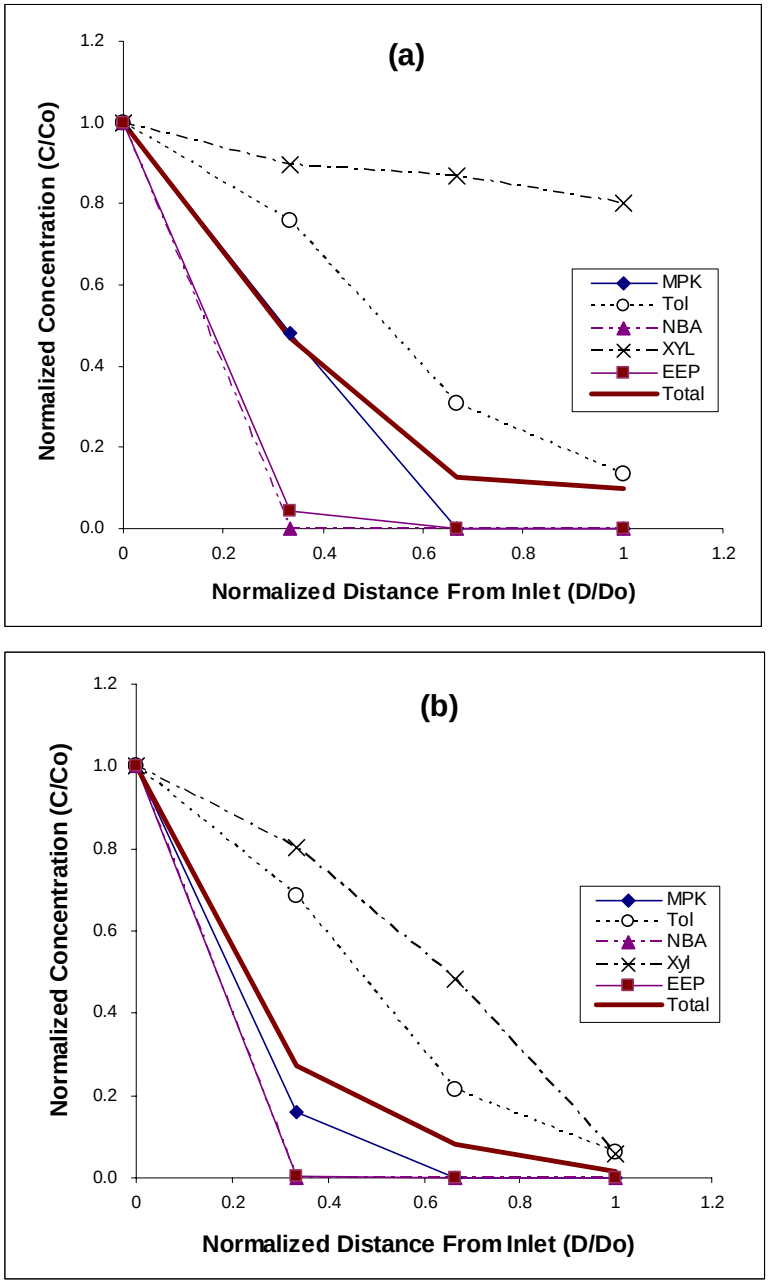


Figure 4-6: Paint mixture removal profile across the SFC on (a) Day 72 and (b) Day 80: (a) one day and (b) nine days after switching from the methyl propyl ketone/toluene feed to the surrogate paint mixture feed.

The SFC, which had been supplied with only toluene for 63 days following startup, sustained high toluene removal (approx. 80 to 90%) whereas toluene removal in the CFC was lower and less stable (varying between approx. 50 and 85%). Xylene removal in both systems was generally much lower (on the order 20 to 50%). Nevertheless, overall VOC removal in both columns remained at approximately 90% throughout bioreactor operation (See Figure 4-7).

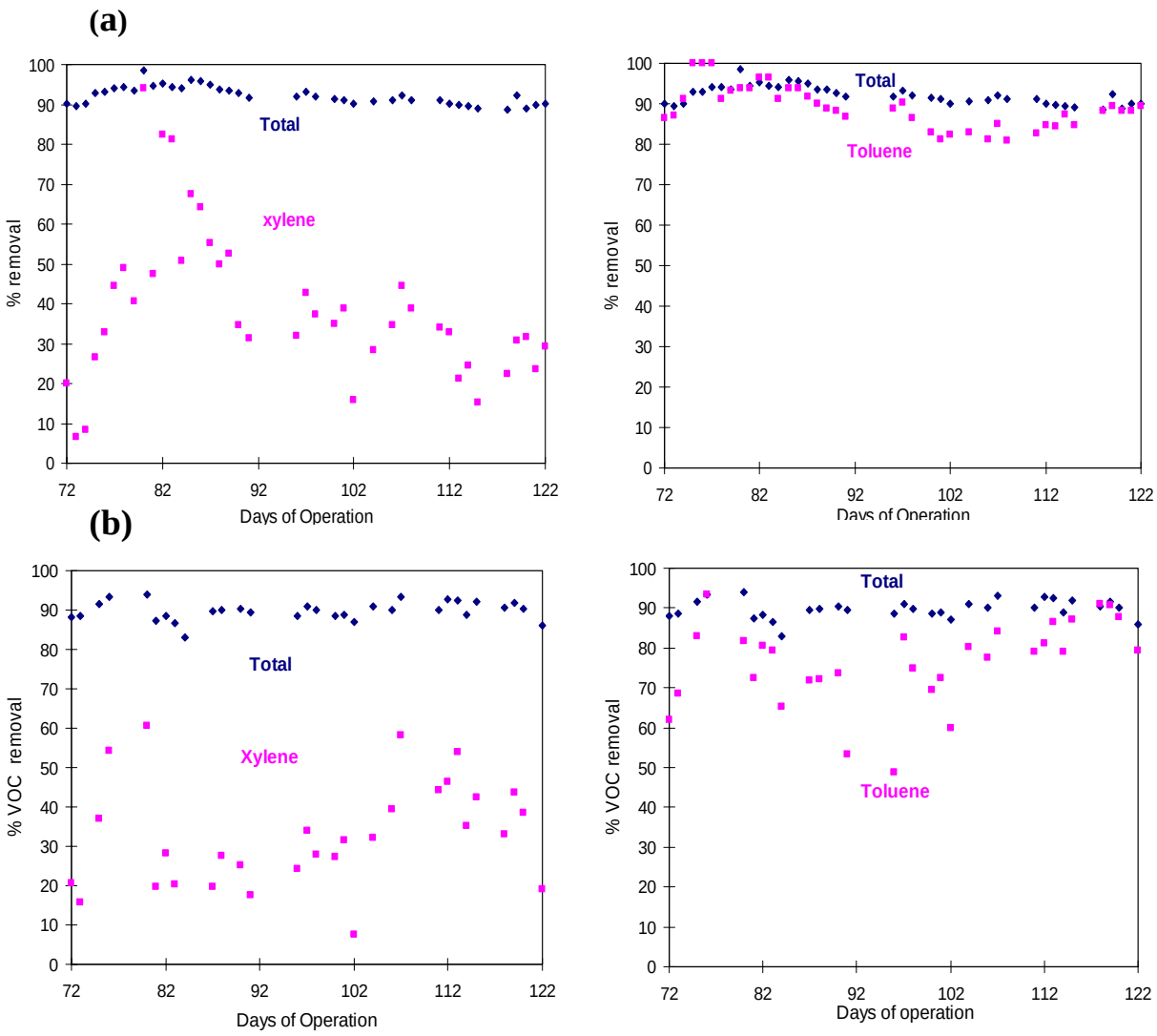


Figure 4-7: Total VOC, toluene and xylene removal efficiencies in the (a) sequentially fed column and (b) continuously fed column from Day 72 to Day 122.

Loss of xylene degradation activity observed in the SFC and CFC columns may have been due to the adverse effect of high salt concentrations in the nutrient solution recirculated in the columns (see further discussion in Chapter 6).

4.3 SUMMARY

The biofilter experiments described in this chapter were conducted to better understand the effects of inoculation method and acclimation history on VOC degradation patterns. The inoculating cultures were individually enriched for each VOC present in the paint VOC mixture to yield a more diverse and robust microbial culture in the bioreactors. In this case, even the continuously fed columns achieved high paint VOC removal efficiencies without significant substrate inhibition but the supply of nutrient was found to be crucial to achieving high removals. In particular, the nitrogen recirculation period must be sufficient to ensure that the nitrogen supply is well distributed through the bioreactor packing material. When the inoculating culture is developed so as to maintain the degradation capacity of the culture for the compounds that are present in low quantities, the sequential feeding strategy did not appear to provide any initial advantage with respect to VOC removal; however, the system ultimately achieved higher aromatic hydrocarbon degrading capacity. Overall, the biodegradation of multiple VOCs is expected to depend on the microbial community present in the bioreactor. As discussed in the next chapter, monitoring the effect of acclimation history on the microbial community may help explain the VOC degradation patterns observed in this task. The other important factor that may affect the microbial community may be nitrogen availability since microbial competition for nitrogen may

affect the composition of the microbial community as well as VOC degradation. This possibility is explored in Chapter 6.

Chapter 5 Effect of Acclimation Method on Biofilm Diversity

Microbial ecological studies have been conducted using denaturing gradient gel electrophoresis (DGGE) techniques to determine if changes in environmental conditions (due to seasonal changes or artificial perturbations) can alter the composition of the microbial population present. Microbial community shifts after environmental changes have been observed in several studies (Donner *et al*. 1996; Santegoeds *et al*. 1997; Teske *et al*. 1996). In our study, it was hypothesized that a similar shift in microbial community could occur in bioreactors that were fed VOC substrates in a different order. Also, spatial variations in the microbial community are expected in biofilters because substrate and nutrient availability are different in each bioreactor section. The DGGE technique is particularly useful when examining time series and population dynamic since it is relatively rapid to perform and many samples can be run simultaneously (Head *et al*. 1998). DGGE is a method based on the electrophoretic separation of PCR-amplified ribosomal gene fragments with the same length but with different sequences on a linear denaturing gradient polyacrylamide gel (Tresse 2002). Separation is based on the decreased electrophoretic mobility of a partially melted double-stranded DNA molecule in polyacrylamide gels containing a linear gradient of DNA denaturants (a mixture of urea and formamide) (Muyzer G. 1998).

Generally, substrate interactions and VOC degradation patterns in biofilters will depend on the microbial community that is established in the column which, in turn may be a function of the acclimation history of the column. During the previous baseline biofilter experiments (Chapter 3), different feeding strategies were found to have an

effect on biofilter performance. In Chapter 4, the effect of acclimation history on VOC removal was investigated and the results indicated that if the column was inoculated with a microbial culture which had been individually enriched for microorganisms capable of degrading each VOC component of the mixture, the initially poor removal of easily degradable compounds might be avoided.

In this Chapter, the effect of the sequential and continuous feeding strategies on the composition of the microbial population in the biofilters was investigated.

For this analysis, biomass samples were collected periodically from the SFC and the CFC described earlier in Chapter 4. To measure the spatial and temporal profiles of the microbial population in each biofilter, microbial samples were collected from the initial inoculation culture as well as from along each biofilter column during operation and analyzed with the DGGE technique. The spatial and temporal diversity of the microbial population in the sequentially-fed and continuously fed biofilters was calculated by using Dice similarity (S_D) and comparing the number of bacterial and fungal bands in each DGGE gel. The Dice similarity approach has been used to delineate the similarity of microbial communities in different samples (Omar 2000; Li 2003). Also, the spatial variation in the microbial community was compared to the VOC degradation pattern observed in each column. Our hypothesis was that even though the same microbial culture was used to inoculate each biofilter, a different microbial community might have established itself in each column depending on the composition of the VOC feed to each bioreactor.

5.1 EXPERIMENTAL METHODS

During the laboratory-scale experiments described in previously in Chapter 4, biomass samples were periodically collected from the bioreactor columns. To provide a basis for comparison, biomass samples were also collected from the microbial culture used to initially inoculate the columns. These samples were analyzed using the DGGE method to characterize the spatial and temporal variations in the microbial population in the bioreactors as a function of acclimation method (Chapter 5) and nitrogen availability (see discussion in Chapter 6). The detailed protocol used for the DGGE analysis of the biomass samples is provided below.

5.1.1 Molecular Biological Analysis

Although biofilters are often presumed to be primarily bacterial, both bacteria and fungi are often present in biofilters. For this reason, the fungal and bacterial populations in the biofilter were monitored separately by using one primer set to amplify 18S rDNA gene fragments from the fungal population and a different primer set to amplify 16S rDNA gene fragments from the bacterial population. The bacterial population was analyzed using the eubacterial 341f primer with a GC clamp and the universal 907r primer (CCTAGGGGAGGCAGCAG and CCCCGTCAATTCATTTGAGTTT respectively) in PCR to amplify the small-subunit 16S rDNA gene fragments. The fungal primers NS5 with a GC clamp and NS6 (AACTTAAAGGAATTGACGGAAG and GCATCACAGACCTGTTATTGCCTC respectively) were used in PCR to amplify 18S rDNA gene fragments from fungi (Muyzer, 1993; Teske, 1996; and White *et al*., 1990). More details of the DGGE method including DNA extraction and PCR amplification are described below.

DNA extraction

Biomass samples for DNA extraction were periodically collected along each biofilter column (Tresse *et al*. 2002). Three foam cubes were collected from each of the three packed sections of the biofilters using sterile tweezers. Each cube (1.5 cm), processed separately, was placed in 50 mL sterile centrifuge tube containing 20 mL sterile HCMM medium (KH_2PO_4 :1.36 g/L, Na_2HPO_4 : 1.42 g/L, KNO3: 3.03 g/L) . Each sample was shaken with 0.5 g of 3 mm glass beads for 5 min and sonicated for 10 min to detach biomass from the foam packing media to the liquid suspension. The polyurethane foam was removed from the centrifuge tube and discarded. The remaining cell suspension was centrifuged at 3000g for 30 min at 4 ^{o}C. The supernatant was then decanted. The cell pellet was washed twice with 20 mL sterilized distilled water. The cell pellet was re-suspended in 2 mL sterilized DI water and then transferred to microcentrifuge tubes (1.5 mL Ependorf microtube). The re-suspended suspension was centrifuged at 1200-1800 g for 5 minutes before removing the remaining supernatant. After 200 μL of a phenol:chloroform (1:1) solution and 0.3 g of glass beads were added to microcentrifuge tubes, the tubes were vortexed at full speed (3000 g) for 5 minutes. 200 μL TE buffer (10mM Tris-Cl, pH7.5; 1mM EDTA) was added and mixed before the tubes were once again centrifuged at full speed for 5 minutes. The top layer was collected into a clean microtube. To coagulate nucleic acids, 1 mL 100% ethanol was added and mixed by inverting. After centrifuging for 3 min at full speed, the pellet was suspended in 200 μL TE buffer with 1.5 μL RNAse and incubated at 37 ^{o}C for 1 hour. Then, 20 μL of 3 M sodium acetate and 0.5 mL of 100% ethanol were added and inverted to mix. DNA coagulates in this step. After another 3 minutes of centrifugation at full speed, the supernatant was removed and mixed with 200 μL of ice cold 70% ethanol.

After centrifugation for an additional 3 minutes, the supernatant was removed and the pellet was dried in a 37 ^{o}C water bath for 30 minutes. The pellet was then re-suspended in 30 μL DI water in an ice bath with occasional flicking. After fully resuspending the pellet in the solution, the DNA was ready for use or storage at - 80^{o}C.

PCR reaction

PCR was performed using a thermocycler (MWG-BioTech, High Point, NC). The 100 μL reaction solutions contained the following: 5 μL of template DNA (~250ng), 1.5 μL of 100 mM magnesium sulfate, 10 μL of 10X buffer, 1.25 μL of Vent polymerase (2 units/μL), 2 μL of 10 mM dNTPs, 5 μL of forward and reverse primers and 70.25 μL of water. The program used was 94^{o}C for 2 minutes followed by 35 cycles of denaturation at 94 ^{o}C for 1 minute, annealing at 53 ^{o}C for 1 minute and extension at 72 ^{o}C for 1 minute followed by final extension at 72 ^{o}C for 10 minutes. The samples were held at 4 ^{o}C until they were removed from the PCR machine. For long term storage, the amplified PCR fragments were stored at - 20 ^{o}C.

DGGE analysis

The DGGE gel consisted of a 20-ml polyacrylamide gel (6% w/v acrylamide) containing a denaturing gradient ranging from 30% to 60% denaturant (where 100% denaturant contains 7 M urea and 40% v/v formamide). Gels were cast using a BioRad Model 475 Gradient Delivery System and then covered by a 5-ml acrylamide stacking gel without denaturant. 20 μL of solution containing 10 μL of PCR product and 10 μL of 2X gel loading dye (0.05% bromophenol blue, 0.05% xylene cyanol, 70% glycerol, BioRad) was loaded into individual wells of the gel. DGGE was performed using a D-Code

Universal Mutation Detection System (Biorad, Hercules, CA) essentially as described by Myers *et al*. (1987) with electrophoresis performed in 1X TAE buffer at 60^{o}C and 65V for 15 h. Following electrophoresis, gels were stained for 10 min with 1 μg/mL ethidium bromide and destained for 30 min with 1X TAE buffer. The gels were then visualized and photographed on a UV transilluminator (Gel Logic 100, Kodak, Rochester, NY).

5.1.2 Bioreactor Operation

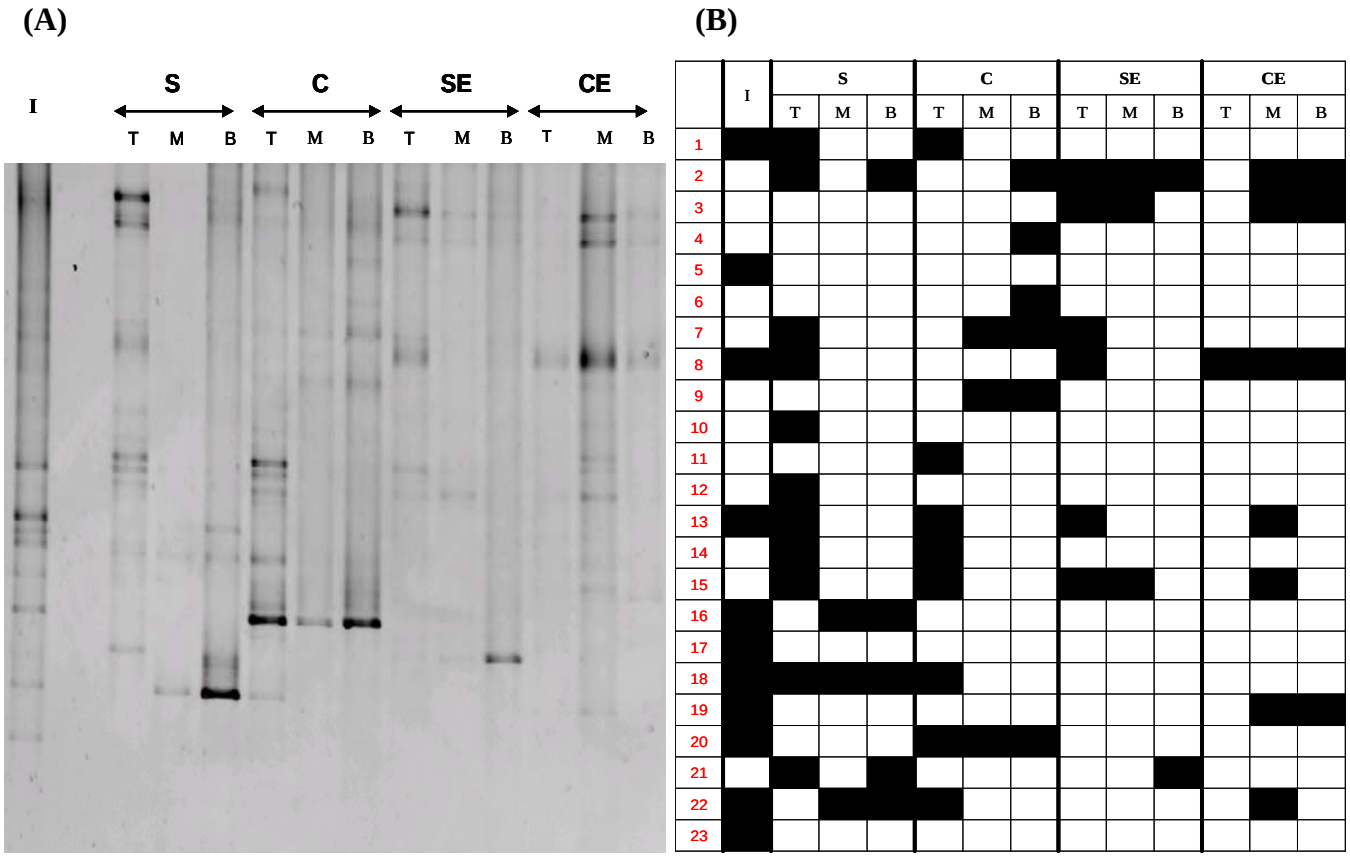
Since directional switching operation was found to improve biomass distribution and activity for continuous long-term use of the bioreactors (Kinney *et al*, 1999), both the sequentially and continuously fed columns were operated in a directional switching (DS) mode for the first 108 days of operation. The first sample for the DGGE (Day 58) was obtained from the each biofilter section when the biofilter was operating in a directionally-switching mode. During directionally-switching operation, the direction of the inlet VOC-laden gas stream through the bioreactor was periodically reversed every 3 days. It was observed that the VOC removal in the biofilter was slightly better when the inlet feed was provided to the bottom of the reactor. It was also hypothesized that unidirectional switching operation would result in the segregation of the microbial community along the column and this might help the aromatic degradation. That is, the bottom section of the column could be specialized for the degradation of easily degradable compounds (e.g. MPK, NBA and EEP) and the upper section might be specialized for less degradable compounds such as toluene and xylene. Thus, following the DS operating period, the sequentially and continuously fed columns were supplied the VOC-laden waste gas from the bottom of the bioreactor for the next 105 days. However, as biomass accumulation in the column increased over long-term operation of the biofilter, nutrient hold-up inside the column significantly increased. This interrupted the

proper operation of the nutrient recirculation pump which was set to supply a given flow rate of nutrients. Thus, during the final 56 days of operation, the inlet of the bioreactors was moved to the top of the bioreactor columns to help move the nutrient solution through the column. As a result, the bioreactors were in top feeding mode when the biomass samples for DGGE analysis were collected on Day 269.

5.2 RESULTS AND DISCUSSION

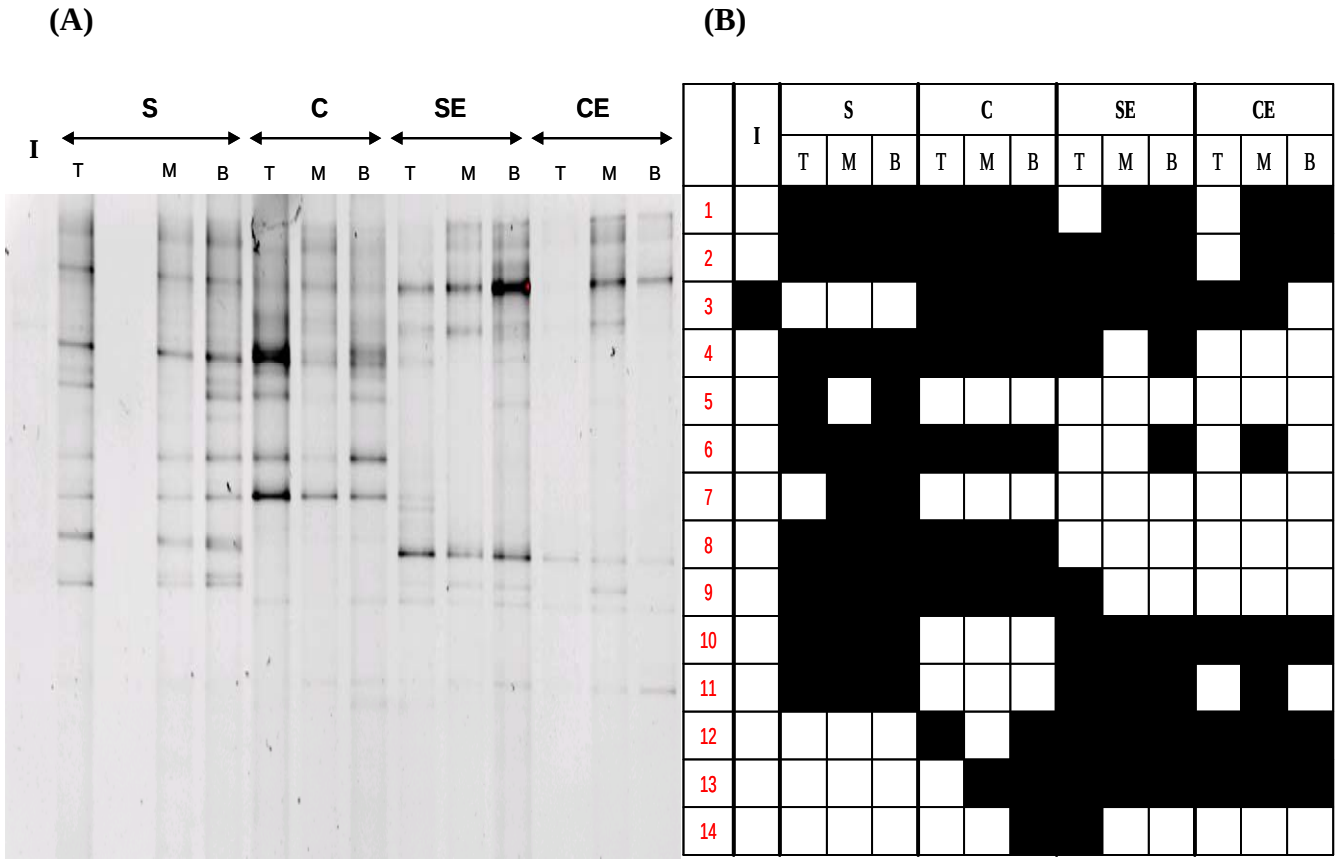
5.2.1 Changes in Microbial Diversity over Extended Periods of Operation

The DGGE gels for the bacterial population and for the fungal population are presented in Figures 5-1 and 5-2, respectively. On each gel, the DGGE profiles are shown for biofilm samples collected from the sequentially and continuously fed columns (described previously in Chapter 4) on Day 58 and Day 269 of operation. For comparison purposes, the DGGE profile of the mixed microbial culture used to inoculate the bioreactors is also provided on each gel.



(I: Inoculum, S: Sequentially fed column on Day 58, C: Continuously fed column on Day 58, SE: Sequentially fed column on Day 269, CE: Continuously fed column on Day 269, T: Top, M: Middle, B: Bottom)

Figure 5-1: DGGE profiles of the bacterial population in the sequentially and continuously fed columns on Day 58 and Day 269 of operation: (a) DGGE gel picture and (b) Digitized banding pattern. Note: Top, Middle and Bottom refer to the location of each of the three packed bed sections in the continuously fed and sequentially fed bioreactor columns.



(I: Inoculum, S: Sequential column on Day 58, C: Continuous column on Day 58, SE: Sequential column on Day 269, CE: Continuous column on Day 269, T: Top, M: Middle, B: Bottom)

Figure 5-2: DGGE profiles of the fungal population in the sequentially and continuously fed columns on Day 58 and Day 269 of operation: (a) DGGE gel picture and (b) Digitized banding pattern.

The DGGE analysis reveals that the initial culture used to inoculate the biofilters was quite diverse and predominantly bacterial (see the lanes identified as "I" in Figures 5-1 and 5-2). Eleven bacterial bands were detected in the microbial culture used to inoculate the bioreactors but only one fungal band. The original source of the inoculum was activated sludge from a local wastewater treatment plant (Walnut Creek Wastewater Treatment Plant, Austin). Although the original activated sludge sample was likely more diverse, the inoculum had been enriched for microorganisms capable of degrading the paint VOCs since only these VOCs were provided as carbon and energy sources during the development of the culture used to inoculate the bioreactors (see Section 4.1.1). Thus, only microbial species which were able to degrade each paint VOC were selectively developed.

By Day 58 of bioreactor operation, three of the 11 bacterial bands in the inoculum had already disappeared in the biofilters. This is not surprising given that the growth conditions in the biofilters were significantly different from those in the liquid batch systems where the initial microbial culture was developed. A well-mixed batch reactor is well suited to supporting the growth of fast-growing, free-living cells since nutrient supply, substrate availability and mass transfer limitations are not a concern in such a system. However, in the vapor phase bioreactor, only microbial species which can attach to the packing media and compete with the other species in the biofilm will survive. The difference between free living and particle-attached communities has been observed often in the literature (Acinas *et al*. 1997; Zhang and Fang, 2000; Tresse *et al*. 2002). Stoffels *et al*. (1998) also demonstrated that a significant shift in the microbial population can occur when a microbial culture is transferred from the original inoculating culture to a bioreactor. In another study, only 50% of the bands

representing the inoculum were detected in the biomass that established itself in a biotrickling filter (Tresse *et al*. 2002).

Another possible reason for the difference between the microbial community found in the inoculum and that found in the biofilm samples collected on Day 58 is that, in our study, the inoculum was developed by combining a series of microbial cultures that had been grown individually with a single VOC as the sole carbon source. By growing the cultures separately prior to combining them, the microbial species in each batch reactor were selectively enriched without competition from microorganisms degrading other carbon sources. However, when the combined culture was transferred to the bioreactor, the microorganisms that thrived in bioreactor would be determined not only by the environmental conditions found in the bioreactor and but also by their ability to compete with microorganisms degrading the other components of the paint mixture.

Spatial distribution across the biofilters

The DGGE pattern for the microbial samples collected from the SFC and CFC biofilters was further analyzed to assess the spatial diversity of the microbial population in the biofilter by comparing the number of bands and Dice's coefficient of similarity (S_D). The number of bands in the DGGE gel provides an indication of how many microbial species were present in the column, whereas the Dice index reveals how similar the microbial communities were that established in each section of the column.

The Dice's coefficient of similarity (S_D) is calculated as follows:

$$S_D = 2n_{ab}(n_a+n_b),$$

where n_{ab} is the number of bands common in both filter-bed location samples, n_a and n_b are the number of bands found in each sample location (Omar 2000; Li 2003). A S_D value of 0 indicates that the samples are completely different, and S_D value of 1 indicates that the samples are identical. The similarity matrix developed using the Dice coefficients for the DGGE band patterns is presented in Table 5-1 for the bacterial population.

Table 5-1: Similarity matrix of bacterial DGGE bands observed in the sequentially and continuously fed columns on Day 58 and Day 269 using the Dice index.

Dice (Czekanowski or Sorenson) Measure														
Case		I	S			C			SE			CE		
			T	M	B	T	M	B	T	M	B	T	M	B
I														
S	T	.36												
	M	.43	**.14**											
	B	.38	**.38**	**.75**										
C	T	.53	.53	.36	.31									
	M	.14	.14	.00	.00	**.18**								
	B	.12	.24	.00	.18	**.14**	**.67**							
SE	T	.24	*.59*	.00	.18	.29	.22	.33						
	M	.00	.29	*.00*	.25	.18	.00	.22	**.67**					
	B	.00	.31	.00	*.57*	.00	.00	.25	**.25**	**.40**				
CE	T	.17	.17	.00	.00	*.00*	.00	.00	.29	.00	.00			
	M	.44	.44	.20	.33	.40	*.00*	.15	.77	.60	.22	**.25**		
	B	.27	.27	.00	.22	.00	.00	*.20*	.60	.57	.33	**.40**	**.73**	

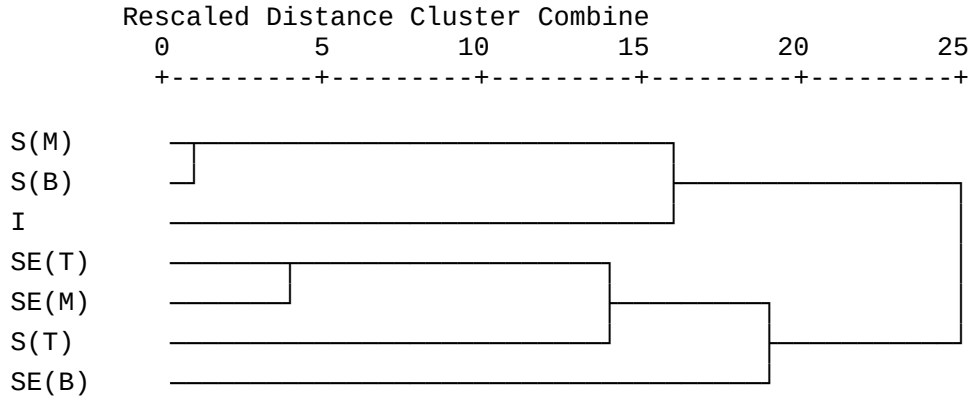
(I: Inoculum, S: Sequential column on Day 58, C: Continuous column on Day 58, SE: Sequential column on Day 269, CE: Continuous column on Day 269, T: Top, M: Middle, B: Bottom, Bold numbers indicate the similarity between bacterial populations present in different spatial locations within the columns. Italic numbers indicate the similarity of bacterial populations present in the same biofilter section on Day 58 and Day 269 of operation)

The DGGE pattern for the microbial samples collected from the SFC and CFC biofilters on Day 58 indicates that more bacterial bands were present in the top section of each of the bioreactors than in the other two bioreactor sections. Eleven bacterial

bands were detected in the top section of the SFC biofilter while three bands were present in the middle and six bands were present bottom section. In the CFC biofilter, eight bands were present in the top section while only three bands were present in the middle section and six bands in the bottom section.

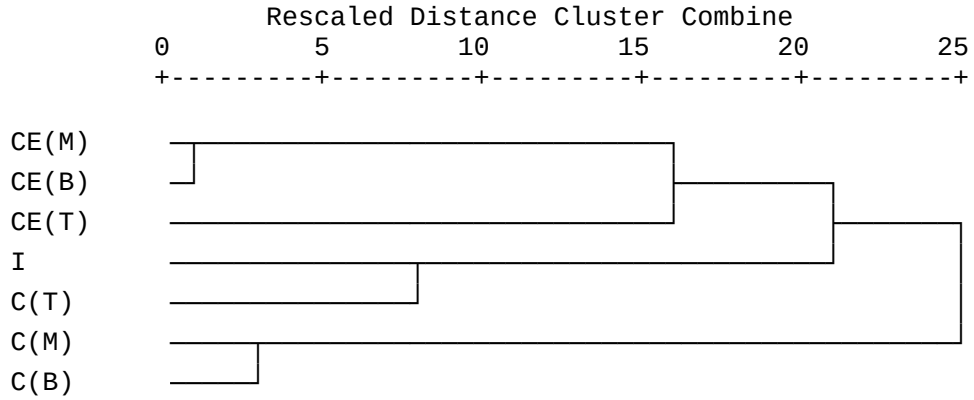
A similarity value of 0.14 was calculated for the bacterial banding patterns observed in the top and middle sections of the SFC on Day 58 indicating that the microbial cultures in these sections were quite different. The microbial populations in the top and bottom sections were also quite dissimilar. In the CFC, the banding patterns indicate that the microbial populations in the middle and bottom bioreactor sections were also quite distinct from the population in the top bioreactor section with similarity values ranging 0.14 to 0.18. In contrast to these results, the DGGE banding patterns for the middle and bottom bioreactor sections were much more similar in both the sequentially and continuously fed columns on Day 58 (a Dice index of 0.75 in the SFC and a Dice index of 0.67 in the CFC).

Dendrograms were also constructed based on the similarity matrix to visualize the similarity of the microbial community in each biofilter section, by using the average linkage (between groups) in Statistical Package for the Social Sciences (SPSS) 10.0. Figures 5-3 and 5-4 shows the dendrograms using the average linkage of the bacterial DGGE bands using cluster analysis. A cluster combined near to 0 of the rescaled distance represents high similarity. All other clusters at a lower similarity combine near 25 on the rescaled distance.



(I: Inoculum, S: Sequential column on Day 58, SE: Sequential column on Day 269, T: Top, M: Middle, B: Bottom)

Figure 5-3: Dendrogram using average linkage (between groups) of the bacterial DGGE bands obtained from the sequential feeding column.



(I: Inoculum, C: Continuous column on Day 58, CE: Continuous column on Day 269, T: Top, M: Middle, B: Bottom)

Figure 5-4: Dendrogram using average linkage (between groups) of the bacterial DGGE bands obtained from the continuous feeding column.

The middle and bottom sections on Day 58 in the sequential feeding column agglomerated at a high similarity in Figure 5-3. Also, Figure 5-4 showed high similarity between the middle and bottom sections on Day 269 and on Day 58 in the continuous feeding column. These dendrograms are consistent with the results determined using the similarity index discussed above.

These results indicate that the top section in each bioreactor section had a more diverse bacterial culture, while the other two sections had a less diverse, but more similar bacterial culture by Day 58 of bioreactor operation. These results suggest that a different bacterial community established itself in the top bioreactor section even though both biofilters were operated in a directional feed-switching (DS) mode during the beginning of biofilter operation. This result is somewhat surprising given that the top and bottom sections each received the same inlet VOC-contaminated waste gas stream every three days during this DS period. In addition, the biomass quantity and available nitrogen levels in the top and bottom sections were similar at this point in the biofilter operation. However, the difference in the bacterial community was reflected in the different VOC degradation patterns observed in these two sections as discussed in Section 5.2.3 below. One possible reason for the difference in the microbial community that developed in the top section versus the rest of the biofilter may be the spray method used to recirculate the nutrients through the bioreactors. A high pressure spray system (BETE Fog Nozzle, Inc., TF type, 10 psi) was used to deliver nutrients to the top of the packing material. There were some empirical evidences that the shear force of the nutrient spray solution detached biomass from the packing media in the column. For instance, after the nutrient solution was reciruculated through the column, a significant amount of biomass was observed in the nutrient reservoir. In addition, visual inspection

indicated that no biomass was present on the top few centimeters of the packed media although it was well established throughout the rest of the column. The shear force from the nutrient spray might have washed the easily detached biomass from the column of the packing media, and, eventually, re-seeded the top section by recycling detached biomass. However, the reason for this difference is not clearly understood.

By Day 269 of operation, the spatial similarity of the microbial population in the biofilters was somewhat higher than that on Day 58. This is probably because only microbial species characterized by good immobilization and long-term stability survived in the column. Thus, fewer bacterial bands and somewhat higher similarity between each biofilter section was observed on Day 269.

Even though only one fungal band was detected in the mixed microbial culture which used to inoculate the bioreactor, several fungal bands were present in each biofilter section on Day 58 and Day 269. In striking contrast to the bacterial results, the number of fungal bands in each biofilter section was quite similar. Table 5-2 presents the similarity matrix for the fungal population in the biofilters. On Day 58 in the sequentially fed column, the similarity in the fungal banding pattern was 0.89 (between the top and middle bioreactor sections), 0.95 (between the top and bottom sections), and 0.95 (between the middle and bottom sections). In the continuously fed column, the corresponding similarity values were 0.88, 0.89 and 0.89 respectively. These results indicate that the fungal population in the biofilter was spatially distributed in a uniform manner across the bioreactor column on Day 58. In contrast to the distinctly different bacterial communities that established themselves along the bioreactor column, the composition of the fungal population was relatively consistent

from section to section. By the end of the experiment (Day 269), the similarity of the fungal banding pattern was 0.75 (top-middle), 0.78 (top-bottom), and 0.88 (middle-bottom) in the sequential column. The corresponding values in the continuously fed column were slightly lower at 0.67, 0.67, and 0.77.

Table 5-2: Similarity matrix of fungal DGGE bands observed in the sequentially and continuously fed columns on Day 58 and Day 269 using the Dice index.

Dice (Czekanowski or Sorenson) Measure														
Case		I	S			C			SE			CE		
			T	M	B	T	M	B	T	M	B	T	M	B
I														
S	T	.00												
	M	.00	**.89**											
	B	.00	**.95**	**.95**										
C	T	.22	.71	.71	.67									
	M	.22	.71	.71	.67	**.88**								
	B	.18	.63	.63	.60	**.89**	**.89**							
SE	T	.20	*.56*	.56	.53	.59	.59	.74						
	M	.25	.50	*.50*	.47	.53	.53	.59	**.75**					
	B	.20	.67	.67	*.63*	.71	.71	.74	**.78**	**.88**				
CE	T	.40	.15	.15	.14	*.33*	.33	.43	.62	.73	.62			
	M	.22	.59	.59	.56	.63	*.63*	.67	.71	.93	.94	**.67**		
	B	.00	.43	.43	.40	.46	.46	*.53*	.57	.83	.71	**.67**	**.77**	

(I: Inoculum, S: Sequential column on Day 58, C: Continuous column on Day 58, SE: Sequential column on Day 269, CE: Continuous column on Day 269, T: Top, M: Middle, B: Bottom, Bold numbers indicate the similarity between fungal populations present in different spatial locations within the columns. Italic numbers indicate the similarity of fungal populations present in the same biofilter section on Day 58 and Day 269 of operation.)

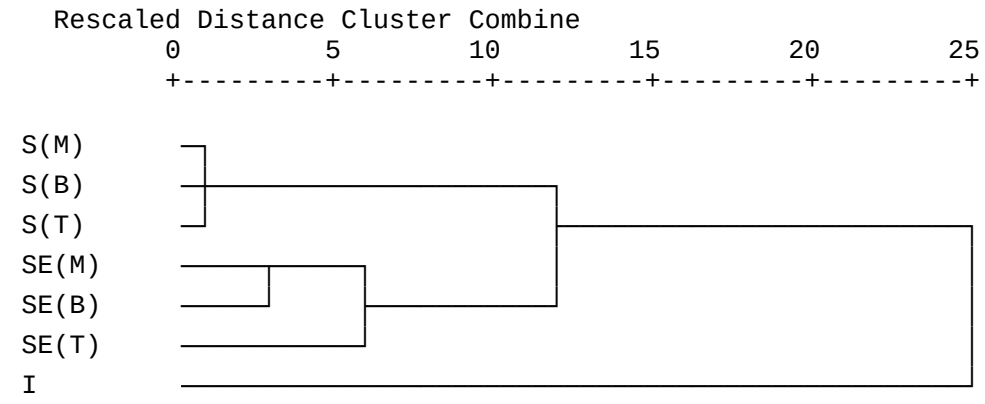


Figure 5-5: Dendrogram using average linkage (between groups) of the fungal DGGE bands obtained from the sequential feeding column.

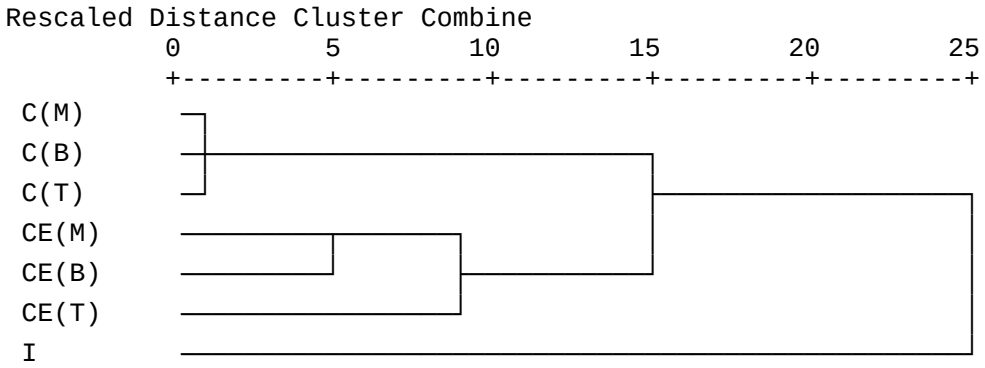


Figure 5-6: Dendrogram using average linkage (between groups) of the fungal DGGE bands obtained from the continuous feeding column.

As can be seen in Figures 5-5 and 5-6, except for the inoculating culture, all the fungal samples from each section at the same time point agglomerated at a high similarity. This result indicates that the fungal species were uniformly distributed along

the column. Comparing the fungal dendrogram to that constructed for the bacterial population, all clusters were constructed at a higher similarity in the fungal dendrogram.

Populations in microbial communities interact in various ways, and these interactions may be either harmful or beneficial. In many cases, the populations interact and cooperate in their feeding efforts, with the waste products of the metabolic activities of some cells serving as the nutrients for others. Thus, the properties of an ecosystem are often controlled to a significant extent by microbial activity. For example, organisms carrying out metabolic processes remove nutrients from the environment and use them to build new cells. At the same time, organisms excrete waste products of their metabolism into the environment. Therefore, over time, a microbial ecosystem can gradually change, both chemically and physically, through living processes (Madigan *et al*. 2000). Even though the biofilter conditions might have initially been favorable to the bacterial population, the makeup of the microbial community ultimately established in the biofilter was influenced to a great extent by the physical and chemical characteristics of that environment. Thus, the environmental conditions in the biofilter were favorable for the growth of fungal species. This could be a reason for the appearance of fungal species in the column. Once the fungal species were present in the column, their morphological properties such as filamentous growth may have enabled them to maintain themselves in the column and resist shear forces. Thus, the fungal species could be stable and similar along the column even over extended operating periods.

Temporal distribution over long-term biofilter operation

The temporal shift in the bacterial and fungal populations over 200 days of biofilter operation was monitored in the SFC and the CFC. Fewer bacterial bands

were present in both bioreactors on Day 269 as compared to those present on Day 58. Assuming that each DGGE band represents a single microbial species, a total of thirteen bacterial species were observed in both the SFC and the CFC at the beginning of the experiment (Day 58). Seven out of the thirteen bacterial bands disappeared and one new band was added by the end of experiment in the SFC, while nine bands disappeared and three new bands were evident in the CFC. A total of seven bacterial bands were present in both of the biofilters by the end of experiments.

When considering the similarity of each biofilter section after 200 days of operation, the bacterial population was significantly different near the beginning of biofilter operation as compared to the population present on Day 269. In the SFC, the temporal variations in the microbial population in the middle section were much greater than the other two sections. That is, in the middle section, no common bands were present on both Day 58 and Day 269 (S_D=0). However, the bacterial population in the top and bottom sections in the sequentially fed column was much more similar over this same time period (0.59 for the top and 0.57 for the bottom). In contrast to the SFC, the bacterial population in the continuously fed column was much less stable over time. That is, none of the bacterial bands present on Day 58 in the top and middle sections were also present on Day 269. In the bottom section, the population was also quite different by Day 269 with a similarity value of only 0.20. However, when considering the total bacterial population present in the entire column, the DGGE band patterns indicate that approximately 46% of the bacterial cultures detected at the beginning of the operation (Day 58) remained at the end of experiment (Day 269) in the SFC and 31% remained in the CFC. These results suggest that the bacterial population was spatially re-distributed along the column over the extended operating period.

The DGGE results indicate that the sequential feeding and continuous feeding strategies yielded a different selective pressure on the bacterial community that established itself in each biofilter. During the start-up period, each biofilter was supplied with a different VOC supply. As a result, five unique bacterial bands (out of total thirteen bands) were present in the SFC and in the CFC biofilter on day 58. That is, 62% of the bacterial species detected in the columns were present in both the SFC and CFC on day 58. However, after 200 days of being supplied the same VOC feed (i.e., the five component surrogate paint mixture), only two unique bacterial bands (out of a total of seven bands) were present in the SFC and CFC. That is, five of the bands detected in the SFC were also present in the CFC 200 days later.

Compared to the bacterial population, the temporal shift in the fungal population was much less significant over time. Comparing biomass samples collected on Day 58 and Day 269 yielded Dice Index values of 0.56 (Top), 0.50 (Middle), and 0.63(Bottom) in the sequentially fed column and values of 0.33 (Top), 0.63 (Middle), and 0.53 (Bottom) in the continuously fed column. When considering the fungal population across the entire column, the DGGE bands suggest that 70% of the fungal species present on Day 58 were still present on Day 269 in the SFC and 60% were still present in the CFC. In addition to being stable with time, the composition of the fungal population in the SFC bioreactor was not that different from the fungal population present in the CFC bioreactor. When comparing the similarity of each biofilter section between the SFC and CFC, a Dice Index value of 0.62 was calculated for the fungal populations in the top bioreactor sections of the SFC and CFC and a Dice Index value of 0.93 and 0.71 were determined when comparing the middle and bottom sections, respectively. If one considers the fungal population established along the entire

column, eight of the 11 fungal bands detected were present in both the SFC and CFC bioreactors on Day 269. These fungal DGGE results indicate that not only was the composition of the fungal population in the biofilters relatively uniform across the bioreactors, it was also quite stable over time. Indeed, it did not appear to be affected significantly by the different VOC- feeding strategies employed in the SFC and the CFC bioreactors.

If one considers only the presence or absence of bands on the DGGE gel, the results discussed above suggest that the sequential vs. continuous feeding strategies had little overall impact on the diversity of the microbial population that developed after 200 days of operation. However, if one considers the relative intensity of the bands in the same lane, the dominant bacterial cultures in the SFC and CFC were likely different. For example, band 20 in Figure 5-2 was the most intense band in the CFC on Day 58 while band 20 was not detected in the SFC. Instead, band 22 was the most intense band in the SFC on that day. Similarly, band 21 was the most intense band in the SFC on Day 269 while this band was not present in the CFC on Day 269. While quantification of a particular species by examining band intensities is difficult, variations in band signal intensity do reveal variations in the abundance of a population in the environment at the order-of-magnitude level (Casamayor 2000). These results therefore suggest that the acclimation method (i.e., sequential feed versus continuous feed) did have an affect on the composition of the microbial community in the column.

5.2.2 The effect of microbial community on VOC degradation patterns

The DGGE analysis demonstrated that the bacterial population community, varied spatially along the column while the fungal community was relatively uniform.

Since this spatial difference in microbial population may affect VOC degradation in the column, it is useful to compare the microbial distributions in the columns to the VOC degradation patterns observed in the biofilters.

During the DS period of operation (Day 1 to Day 108), it was observed that the VOC removal profile was quite different depending on the direction the gas feed was passed through the column even though the biofilter achieved similar overall VOC removals regardless of the feed direction. Figures 5-7 and 5-8 show the VOC removal profiles in the SFC and CFC respectively on Day 58. The VOC degradation pattern across the column was much more different in the CFC than in the SFC. This difference could be explained by the dissimilarity in the microbial population present in each section of the CFC. The top and bottom sections had three common bacterial bands in the SFC (S_D=0.38) on Day 58 but only one common bacterial band in the CFC (S_D=0.14) on Day 58. These results suggest that a significantly different bacterial population became established in the top and bottom bioreactor sections in the bioreactors and the variation in the bacterial population was much greater in the CFC. This difference in the bacterial population was reflected in the different VOC degradation patterns observed when the bioreactor inlet was at the top of the column versus at the bottom of the column. For example, the overall VOC removal and degradation of the aromatic compounds was greater in the inlet (bottom) bioreactor section when the inlet feed to the CFC was placed at the bottom of the bioreactor. Despite these different VOC degradation patterns, however, the fungal population present in the top and bottom sections of the SFC and CFC were nearly identical (i.e., a Dice Index value of 0.95 in the SFC and 0.89 in the CFC). These results suggest that the variations observed in the VOC degradation patterns were not a direct function of the fungal population present in the column but rather more related to the bacterial

population present. Thus, the fungal population did not seem to be directly involved with VOC degradation. Rather, the fungal population may have been acting as a predator or been consuming secondary products from the metabolic activity of the bacterial population. If this is the case, it would explain why the composition of the fungal community was relatively uniform across the column since it may have not been using the VOCs directly as their carbon and energy source.

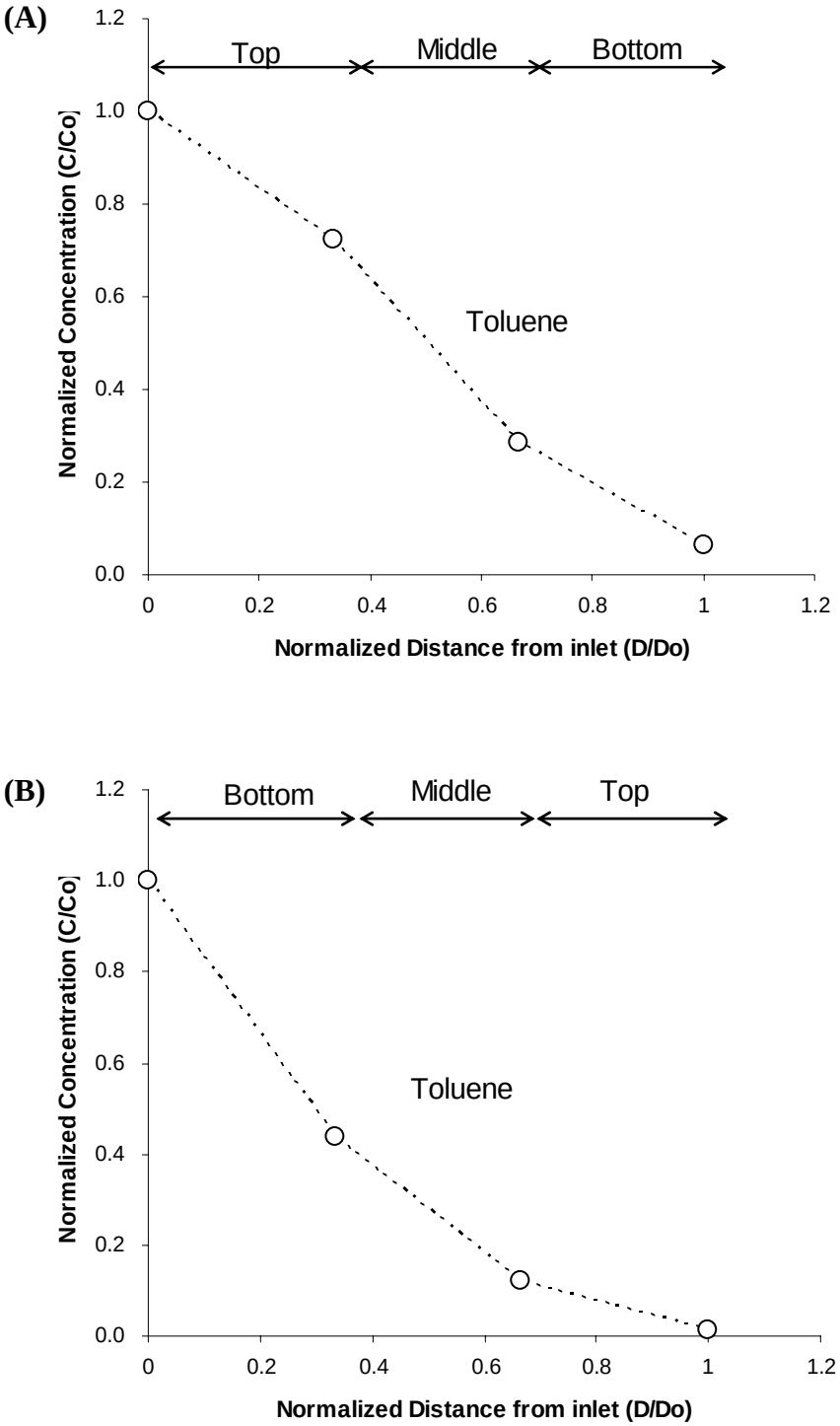


Figure 5-7: VOC removal profile across the sequentially fed column on (a) Day 55 (Top feeding) and (b) 58 (Bottom feeding). Note: top and bottom feeding was alternatively applied to the biofilter every three days. Toluene: 34 g-C/m^3-hr.

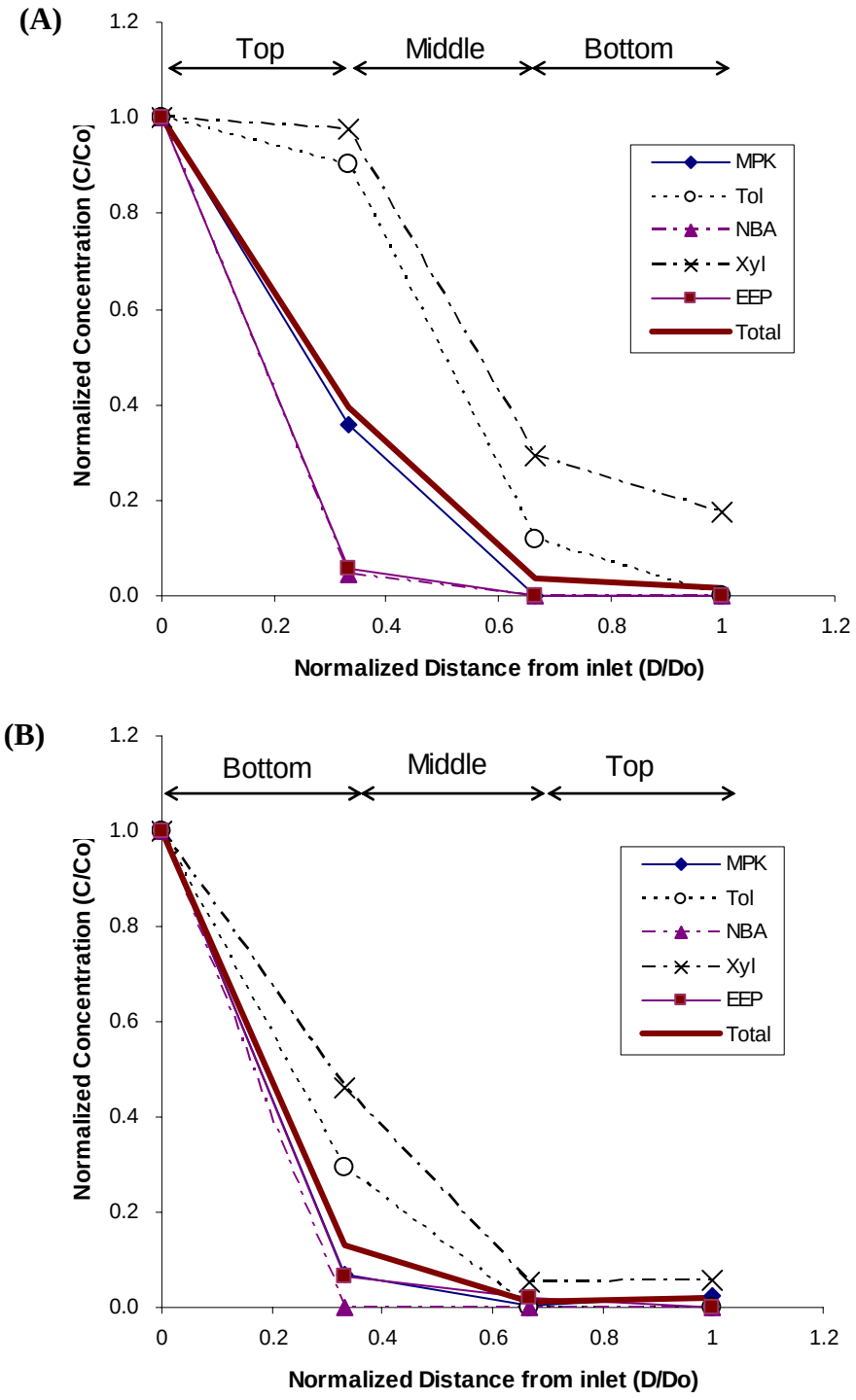


Figure 5-8: VOC removal profile across the continuously fed column on (a) Day 55 (Top feeding) and (b) 58 (Bottom feeding).

Figure 5-9 shows the VOC removal profile along the biofilters on Day 269 when the biofilter was operated with a top VOC feed. On Day 269 in the continuously fed column, no common bacterial bands were detected in the top and middle sections. The similarity of the continuously fed column on between on Day 58 and 269 was 0 (top), 0 (middle), and 0.2 (bottom) for the bacterial population and 0.33 (top), 0.63 (middle), and 0.53 (bottom) for the fungal population. The bacterial community developed in each biofilter section on Day 269 was quite different from those on Day 58 while the fungal community on Day 269 was relatively similar to those on Day 58. The VOC removal profile in the CFC on Day 269 was rather similar to the one in the SFC on Day 269; the similarity of these two columns was 0.29 (top), 0.60 (middle), and 0.33 (bottom) for the bacterial population and 0.62 (top), 0.93 (middle), and 0.71 (bottom) for the fungal population. This result indicates that the VOC removal was strongly dependant on the microbial culture present in the column and the spatial distribution of the microbial community was reflected in the VOC removal profiles observed across the column.

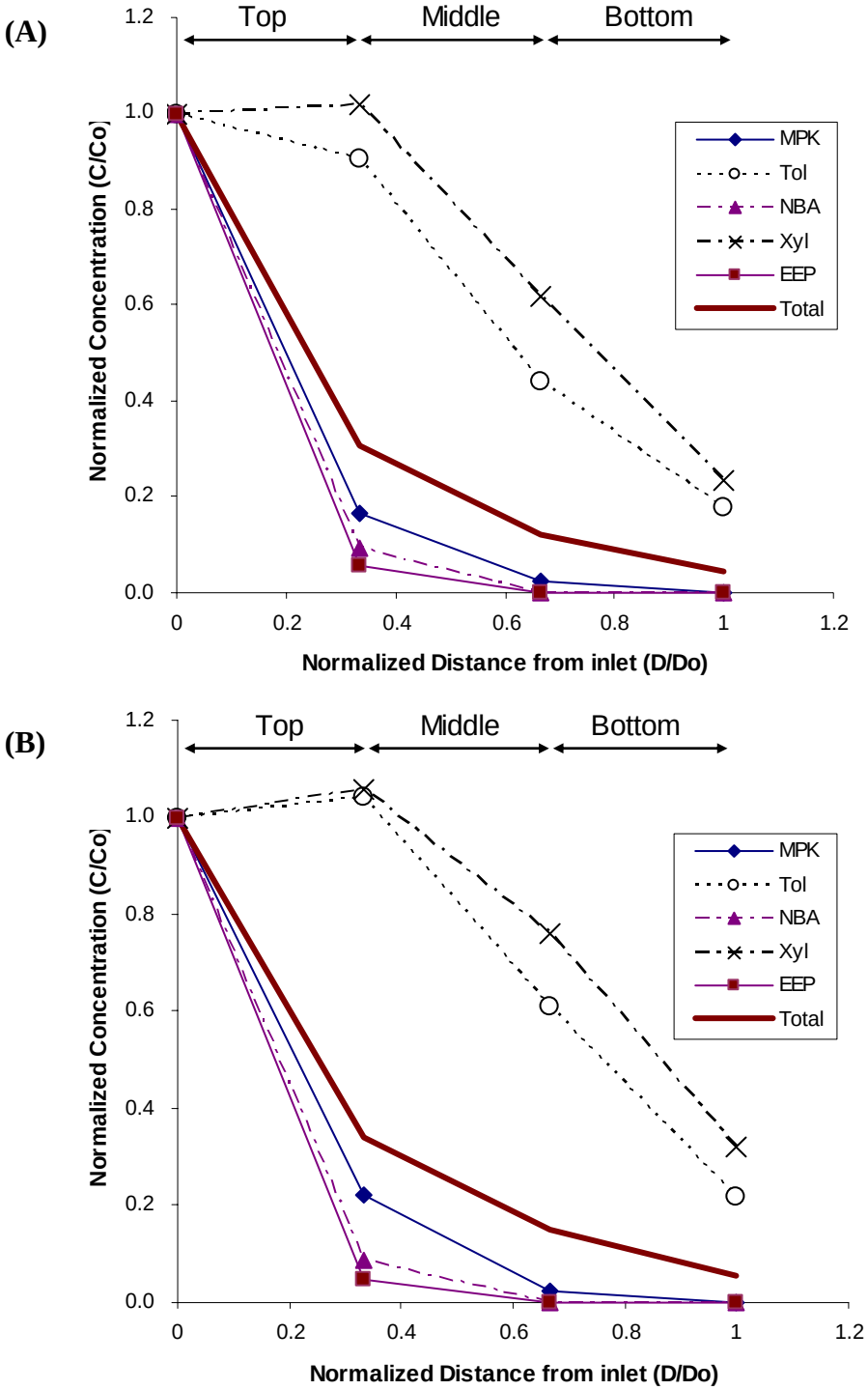


Figure 5-9: VOC removal profile across both (a) the sequentially and (b) continuously fed column on Day 269. Note: only top feeding was applied to the biofilter for past 46 days.

5.3 SUMMARY

Microbial samples were periodically collected from the each biofilter section during the previous laboratory-scale experiments described in Chapter 4. These samples were analyzed using the DGGE technique to investigate the spatial and temporal diversity of the microbial community established in the biofilter. The DGGE banding pattern results indicate that the culture used to inoculate the bioreactors was predominantly bacterial. Some of these initial bacterial species disappeared shortly after being transferred from a well-mixed batch system to the bioreactor possibly due to the different environmental conditions and microbial competition encountered in the biofilm.

The sequential and continuous feeding strategies had little overall impact on the diversity of the microbial population present in the column over extended periods of operation. However, if one considers the composition of the microbial population in each biofilter section over time and compares the relative intensity of microbial bands in each DGGE gel, different acclimation histories did have an effect on the composition of the bacterial community in the biofilter. Generally, the bacterial population present in each biofilter section was distinct from the bacterial community present in the other bioreactor sections. However, if one considers the bioreactor as a whole, the composition of the bacterial community established in the column was fairly stable over extended operating periods indicating that the bacterial species were re-distributed within the column over time. In contrast, the fungal species in the column were relatively uniform across the column and also quite stable over time.

The VOC removal profile seemed to be highly dependant on the microbial community present, especially the bacterial population present in the column. Nitrogen availability may also impact microbial competition in the column, which in turn, may

result in a shift in the microbial population present. Thus, the effect of nitrogen availability on VOC degradation and the composition of the microbial community is investigated in the next chapter.

Chapter 6 Effect of Nitrogen Availability on the Degradation of Paint VOCs

Several studies have been conducted to investigate the effect of nutrients on the performance of biofilters treating single pollutants (Rihn 1997; Gribbins 1998; Jorio 2000; Delhomenie 2001; Moe 2001). However, relatively little work has been completed to assess how nutrient levels affect the degradation of VOC mixtures. Holubar *et al.* (1999) investigated the effect of nitrogen limitation on both a young and a mature steady-state biofilm in a trickle-bed filter treating toluene and *n*-heptane. They demonstrated that while biofilm growth responded strongly to the amount of nitrogen available, the hydrocarbon degradation efficiency reached a maximum of 60% and could not be increased by further addition of nitrogen (Holubar 1999). Only the overall hydrocarbon degradation efficiency was determined in this study and the effect of nitrogen limitation on the degradation pattern of each chemical in the biofilter was not considered. Since aircraft and automotive paint spray operations generate waste gas streams containing a complex mixture of easily degradable and relatively recalcitrant compounds, nutrient levels may have a different effect on the degradation of each chemical in the mixture. The sensitivity of each chemical to nutrient limitation may result from microbial competition within the biofilm for the limited nutrients. For example, some microbial species adapted to low nutrient concentrations may out compete other species that require nutrient rich conditions and, this in turn may affect the biodegradation pattern observed for a mixture of carbon substrates.

A series of experiments were conducted to determine the effect that nitrogen limited conditions have on VOC biodegradation patterns and the composition of the microbial community in the SFC and CFC biofilters. After each biofilter reached

steady removal of the paint VOC mixture without nitrogen limitation, the effect of nitrogen limitation on the VOC degradation patterns was investigated by removing the nitrogen source from the nutrient solution. In addition, biofilm samples were collected for DGGE analysis to ascertain how N-limitation affected the diversity of the microbial population. In the next phase of experiments, the minimal nitrogen level required for effective paint VOC removal was determined by varying the nitrogen concentration and nutrient supply frequency. Several studies have revealed that nitrogen can become kinetically limiting in biofilters even when a stoichiometric excess of nutrients is supplied (Moe 2001; Rhin 1997). Hence, it is important to measure how much nitrogen is actually present in the biofilter biofilm rather than just determining how much nitrogen is supplied to the biofilter.

6.1 EXPERIMENTAL METHODS

The same SFC and CFC biofilters described previously in Chapters 4 and 5 were used for the nitrogen limited experiments. These biofilters had been operated under nitrogen rich conditions for approximately 120 days and had reached steady state removal of the paint VOC mixture. The effect of nitrogen limitation on VOC removal in the biofilter was investigated from Day 123 to Day 163 by removing all the nitrogen-containing components from the nutrient media solution (see Table 3.3 except for KNO_3 (20.2 g/L) and $(NH_4)_2HPO_4$, (1.32 g/L)). When inorganic nitrogen was not detectable in the column during nitrogen limited experiment, media samples were sent to a commercial lab (EMSL Analytical, Westmont, NJ) for organic nitrogen analysis.

After the biofilter had been operated under nitrogen limited conditions for 40 days, nitrogen addition to the nutrient solution was resumed on day 164 to investigate

the minimal nitrogen level necessary to maintain high VOC removal efficiencies. In these experiments, a range of nitrogen concentrations and spraying frequencies were investigated as detailed in Table 6-1.

Table 6-1: Experimental conditions for each operating period during the minimum nitrogen level experiments.

Period	Day	Concentration		Nutrient Spraying Frequency
		Nitrate	Ammonia	
I	164 – 184	20.2 g/L KNO_3	3.96 g/L $(NH_4)_2HPO_4$	30 min twice per day
II	185 – 191	10.1 g/L KNO_3	3.96 g/L $(NH_4)_2SO_4$	30 min twice per day
III	192 – 198	10.1 g/L KNO_3	3.96 g/L $(NH_4)_2SO_4$	30 min once a day
IV	199 – 205	5 g/L KNO_3	3.96 g/L $(NH_4)_2HPO_4$	30 min once a day

6.2 RESULTS AND DISCUSSION

6.2.1 Nitrogen Limited Experiments.

Figure 6-1 summarizes the overall VOC removal efficiency as well as the toluene and xylene removals observed over 40 days of nutrient limited conditions in the sequentially fed and continuously fed biofilters. The nitrogen levels measured in biomass samples collected from the foam packing are also plotted in Figure 6-1.

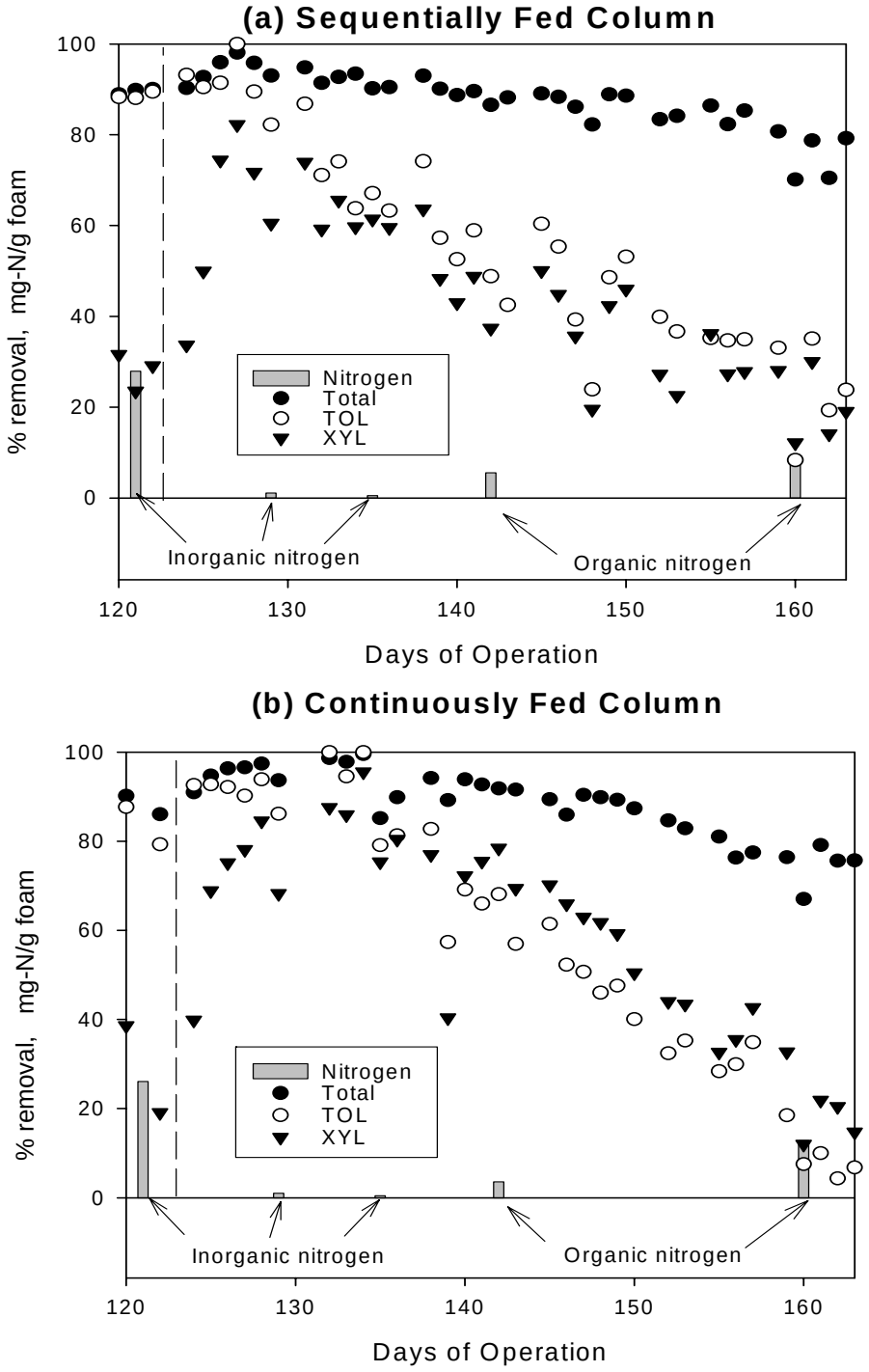


Figure 6-1: Effect of nitrogen limitation on VOC removal in the (a) SFC and (b) CFC biofilters. (Note: The dashed line indicates when nitrogen was removed from the nutrient solution)

As can be seen in Figure 6-1, six days after removing the nitrogen from the nutrient solution on Day 129, the inorganic nitrogen concentration (NO_3-N + NH_4-N) in the packing media quickly dropped. Interestingly, the xylene and toluene removal increased right after the nitrogen source was eliminated from the nutrient solution. Due to the need to limit sampling events to minimize disturbance to the biofilter columns, the nutrient level was not determined during the period when high removals of xylene and toluene occurred in both columns. However, it is suspected that the high salt concentration in the nutrient solution may have inhibited the activity of the aromatic degrading microorganisms. When the nitrogen-containing components were removed from the nutrient solution, the ionic strength of the solution was greatly reduced which may have been the reason for the initial improvement in the aromatic hydrocarbon degrading capacity of the biofilters. However, as the inorganic nitrogen in the column became severely depleted, the toluene and xylene removals gradually decreased. On day 135, the inorganic nitrogen concentration was almost completely depleted. Even after complete depletion of inorganic nitrogen, methyl n-propyl ketnoe (MPK) breakthrough did not occur for another two weeks probably because the microorganisms were utilizing recycled organic nitrogen (Song *et al.*, 2003). On days 142 and 160, the inorganic nitrogen concentration in the biofilm was measured to see how much inorganic nitrogen was left in the column. Interestingly, the organic nitrogen level was somewhat higher on day 160 than that observed on day 142. Finally, after approximately 35 days of biofilter operation under nitrogen limited conditions, breakthrough of MPK was also observed. However, n-butyl acetate (NBA) and ethyl-3-ethoxy-propionate (EEP) were still completely degraded along the column since they are readily degradable and constitute a relatively small fraction of the total VOC concentration entering the columns (e.g., 25% of the total VOC feed). It is rather

interesting to note that the order of breakthrough of each of the paint VOCs under nitrogen limited conditions in this experiment was the exactly reverse of the order in which the VOCs were found to be degraded when nitrogen rich conditions were being established in the column during the start up period.

6.2.2 Minimum Nitrogen Level

Even though the VOC removal efficiency eventually decreased under nitrogen limited conditions, even higher VOC removals were achieved immediately after removing the nitrogen source from the nutrient solution during the nitrogen limitation experiments. This result suggests that high concentrations of nitrogen in the nutrient spray solution may adversely affect microbial activity possibly due to the presence of high salt concentrations. The aromatic degrading microbial population seemed to be the most sensitive to nitrogen concentration. Thus, it would be useful to investigate the minimal nitrogen level necessary to maintain high VOC removal efficiencies. To this end, a range of nitrogen concentrations and spraying frequencies were investigated in a series of experiments (see Table 6-1).

In these experiments, nitrogen addition to the nutrient solution was resumed on day 164 after more than 40 days of operating under nitrogen limited conditions. As soon as the inorganic nitrogen source was reintroduced to the biofilter, the VOC removal efficiency increased immediately. Toluene and xylene removal efficiency increased with nitrogen addition up to a point. The VOC removal efficiencies observed during the minimum nitrogen level experiments are shown in Figure 6-2. Figures 6-3 and 6-4 present the VOC removal profile observed across the SFC and CFC biofilters as

well as the inorganic nitrogen levels measured in each section during each nutrient feed period.

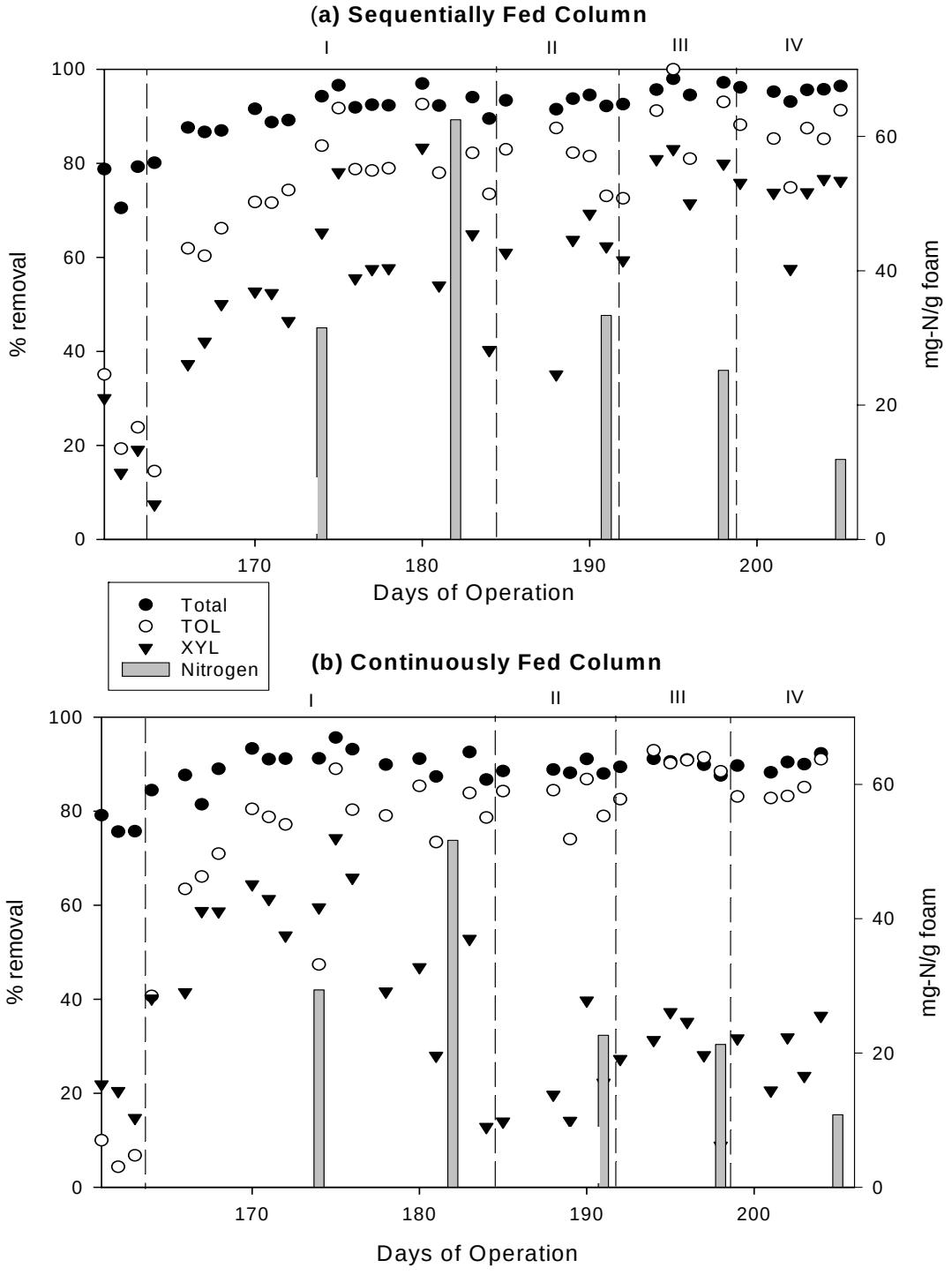


Figure 6-2: Effects of nitrogen level on VOC removal efficiencies. (Note: Roman numbers (I, II, III and IV) correspond the nutrient feed periods delineated in Table 6-1; the nitrogen level before Period I was negligible)

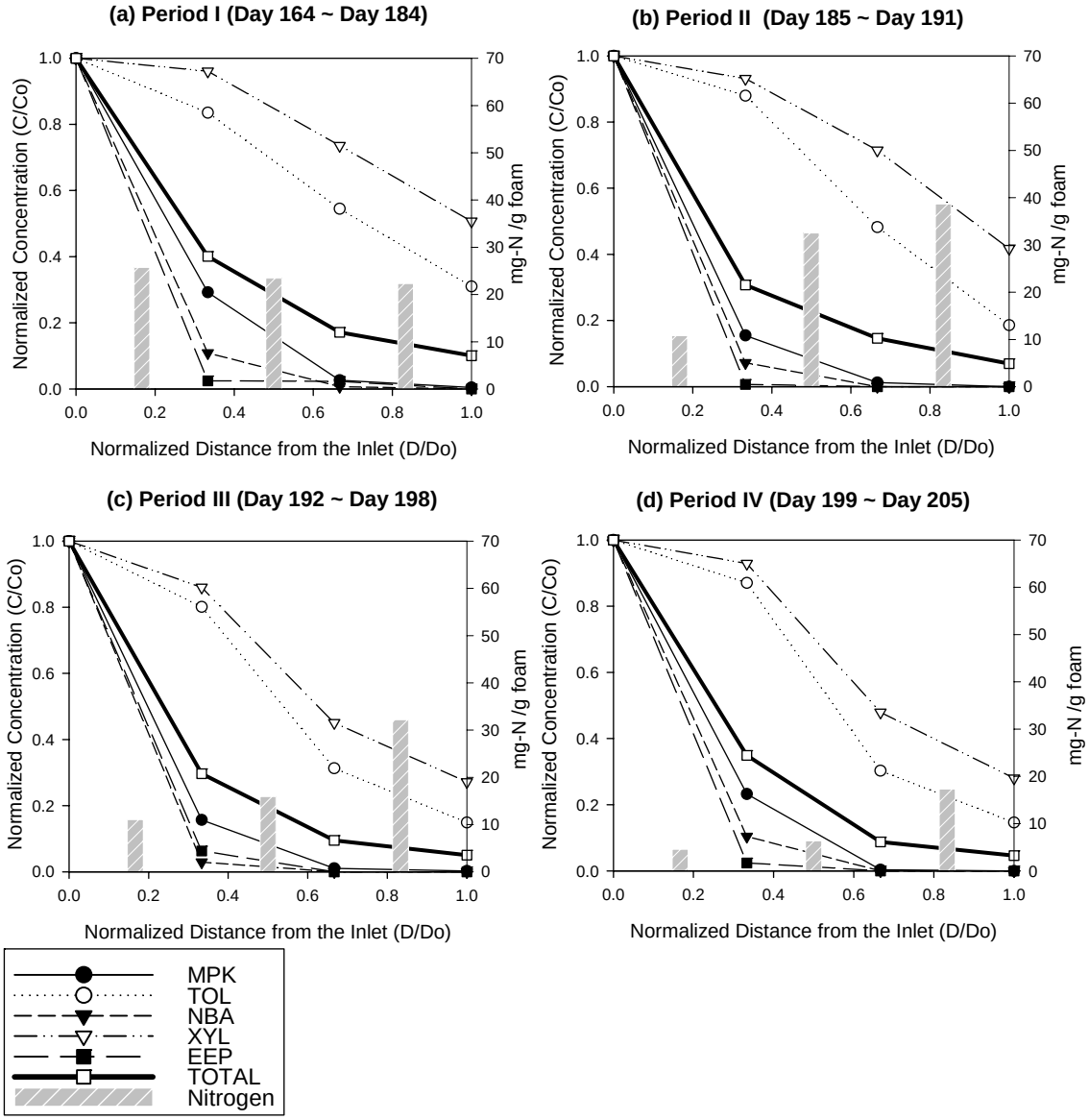


Figure 6-3: VOC removal profiles during the nitrogen limited experiments in the sequentially fed column.

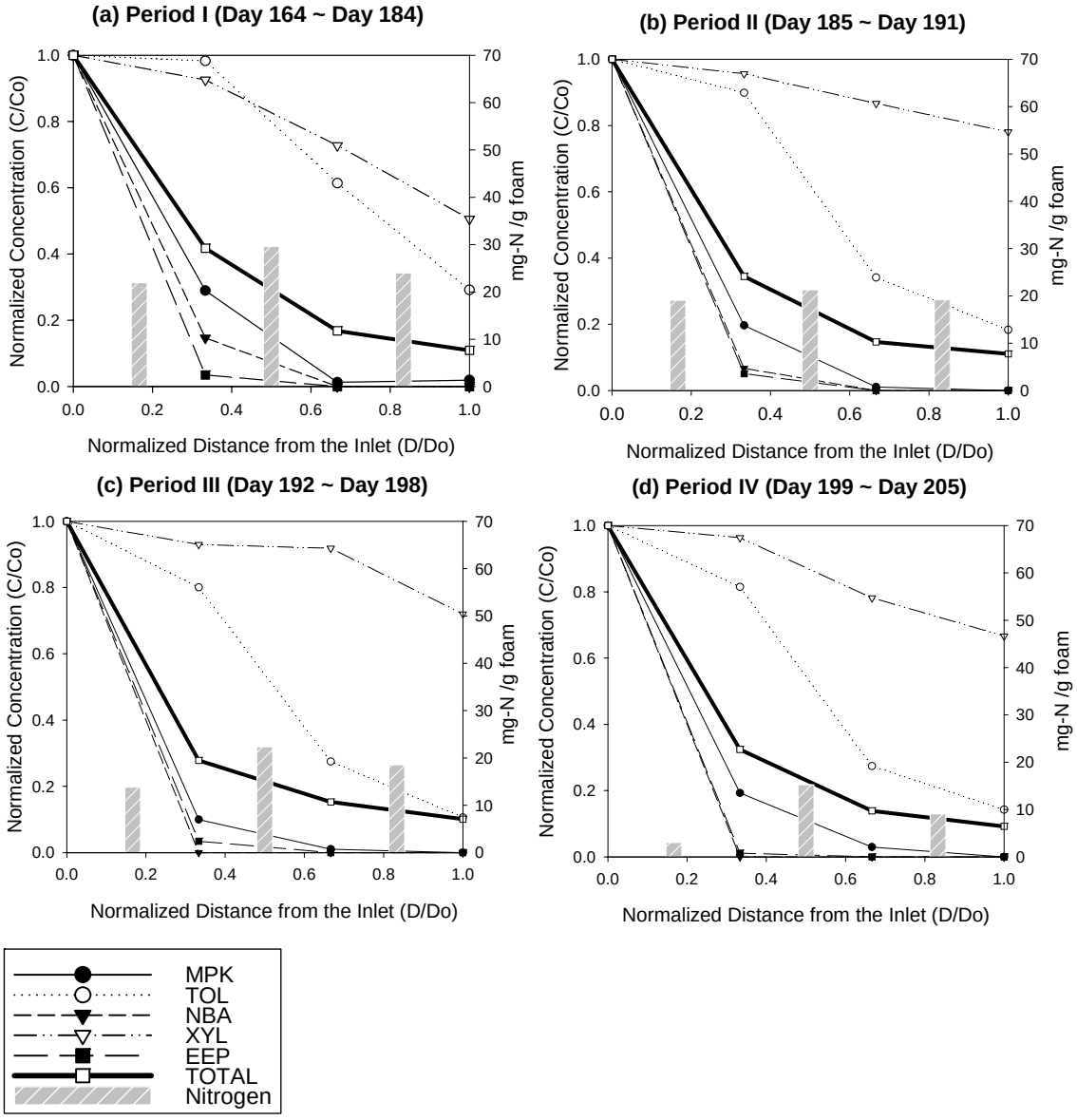


Figure 6-4: VOC removal profiles during the nitrogen limited experiments in the continuously fed column.

As observed in the previous experiments, the xylene removal efficiency decreased when the nitrogen levels in the packing media were too high during period I. Unfortunately, the nitrogen level that is detrimental for xylene removal could not be determined in this experiment due to limited sampling points. However, when the nitrogen concentration was decreased in period II, the xylene removal efficiency began to increase. In the sequentially fed column, approximately 80 % removal of xylene was achieved. However, the recovery of xylene removal efficiency in continuously fed column was not as high as that observed in the sequential fed column. The CFC ultimately achieved approximately 35% removal of xylene by the end of this experiment (Period IV).

As mentioned in the previous start-up experiments, VOC removal efficiency is not solely a function of nutrient level but it is also related to the biomass quantity established on the biofilter packing material. On Day 16 and Day 205, the biofilters had similar inorganic nitrogen levels actually retained in the biofilm. However, the total VOC removal efficiency was approximately 70% on Day 16, and greater than 90% on Day 205. The major difference between these two sampling points was the quantity of biomass that had established on the foam media in the each column. The biomass quantity on Day 16 was 10% of that present on Day 205. These results indicate that insufficient biomass was present in the immature biofilm present on Day 16 to completely degrade the inlet VOC mixture. More organic nitrogen was also likely available for use by the microorganisms on Day 205 even though the inorganic nitrogen availability was similar between the two time periods. Song *et al*. (2003) demonstrated that as much as 56% of the nitrogen needed by the microorganisms may be recycled from organic matter within the biofilm (Song, *et al*, 2003).

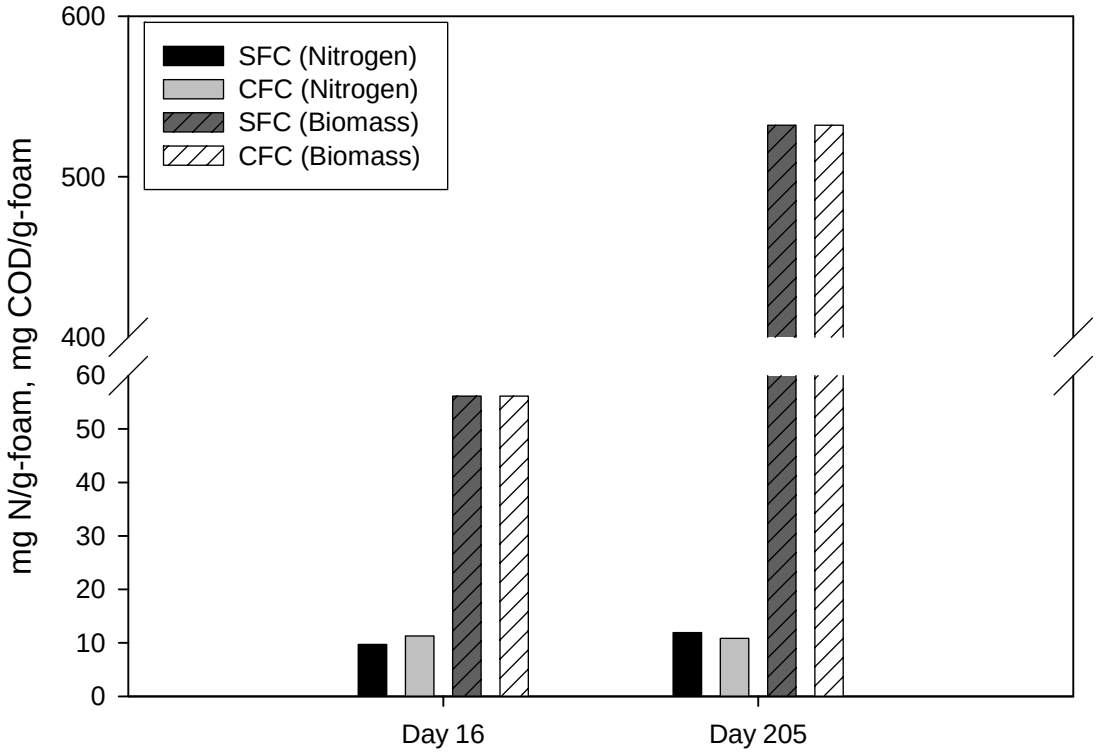


Figure 6-5: Comparison of nitrogen and biomass between Day 16 and Day 205 of operation

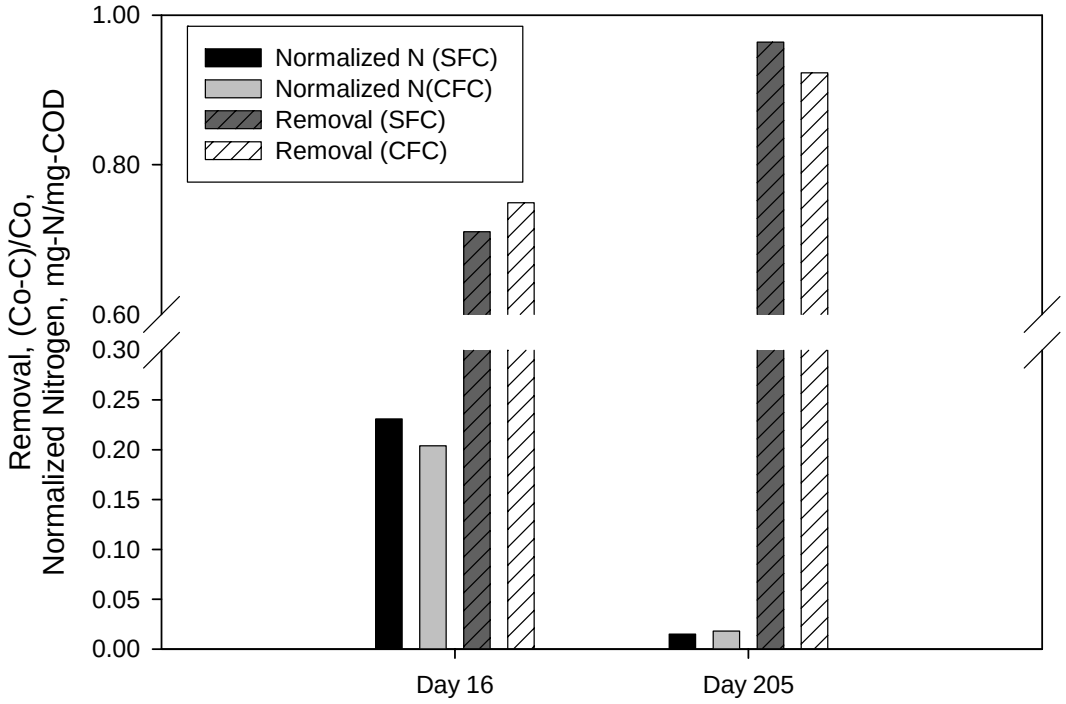
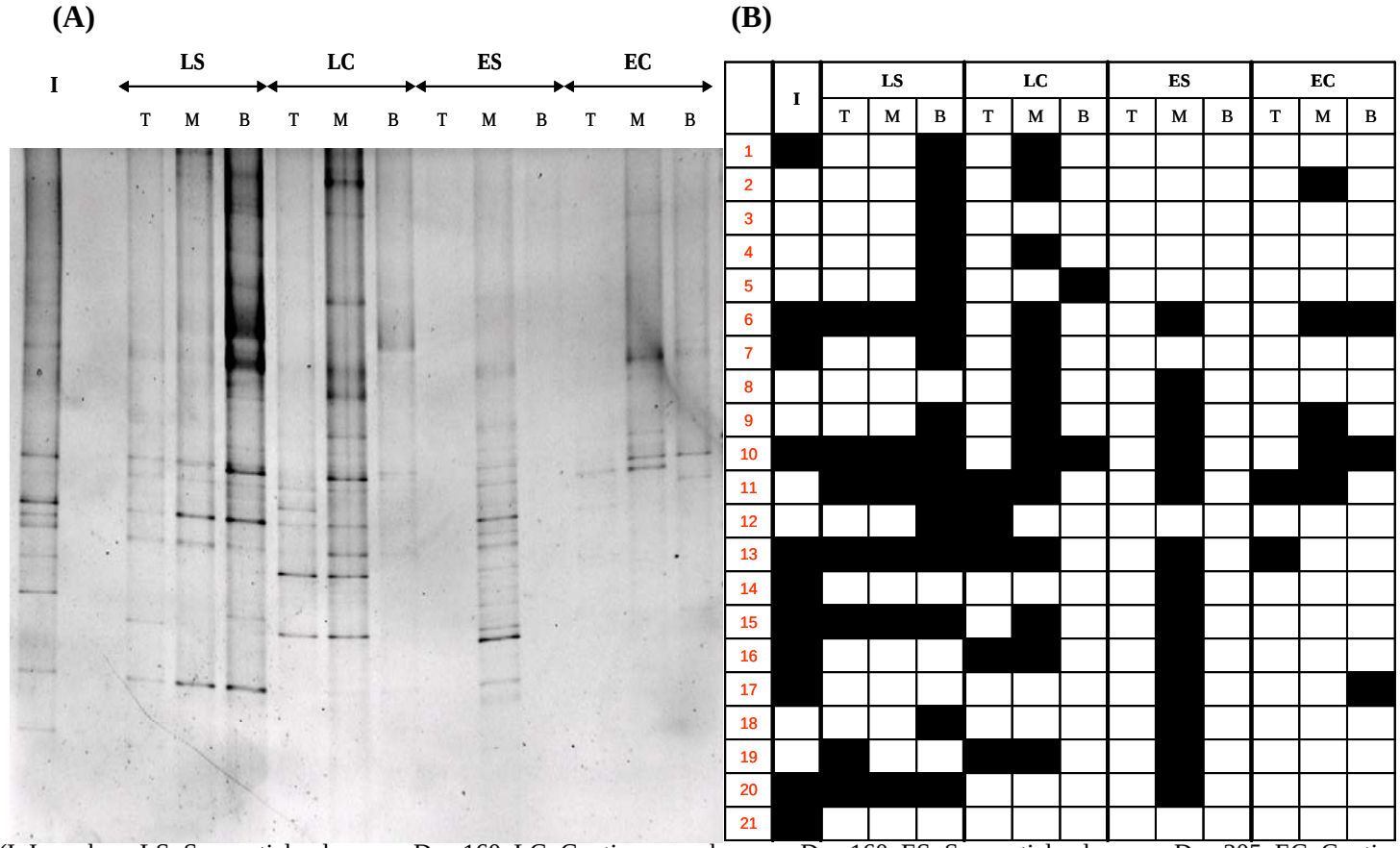


Figure 6-6: Comparison of normalized nitrogen (by biomass amount in the packing media) and VOC removal between Day 16 and Day 205 of operation. Note: (Co: inlet VOC concentration, C: outlet VOC concentration, normalized N: inorganic nitrogen (mg-N/g-foam)/ biomass amount (mg-COD/g-foam))

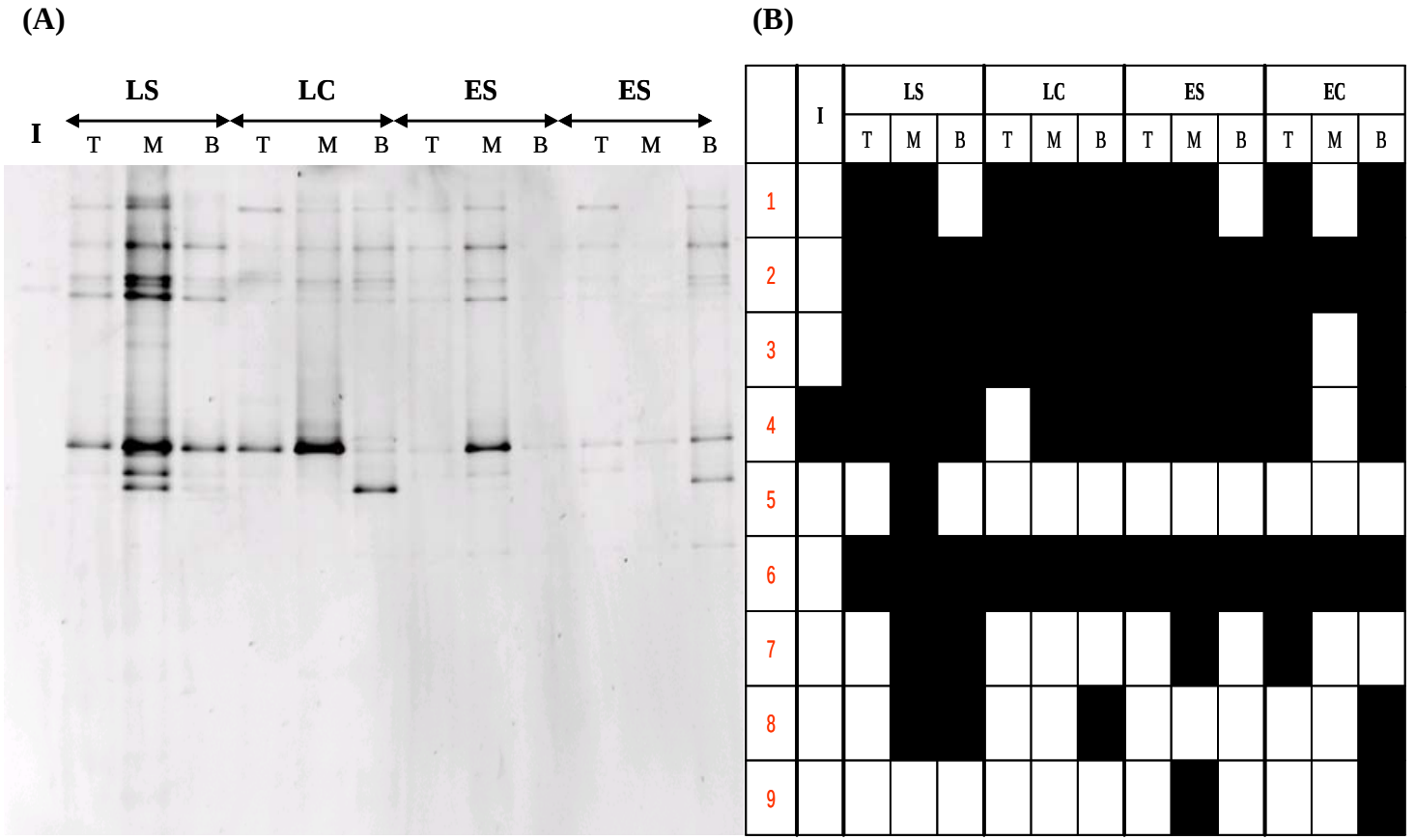
6.2.3 Microbial Diversity under Nitrogen Limited and Nitrogen Rich Conditions

To investigate the effect of nitrogen on the microbial population, the microbial population was monitored in the biofilter under nitrogen limited conditions (Day 160) and nitrogen rich conditions (Day 205). The resulting DGGE gels for the bacterial and fungal populations on Day 160 (nitrogen limited conditions) and Day 205 (nitrogen rich conditions) are presented in Figures 6-7 and 6-8, respectively.



(I: Inoculum, LS: Sequential column on Day 160, LC: Continuous column on Day 160, ES: Sequential column on Day 205, EC: Continuous column on Day 205, T: Top, M: Middle, B: Bottom)

Figure 6-7: DGGE profiles of the bacterial population in the sequentially and continuously fed columns on Day 160 (nitrogen rich conditions) and Day 205 (nitrogen limited conditions): (a) DGGE gel picture and (b) Digitized banding pattern. Note: Top, Middle and Bottom refer to the location of each of the three packed bed sections in the continuously fed and sequentially fed bioreactor columns.



(I: Inoculum, LS: Sequential column on Day 160, LC: Continuous column on Day 160, ES: Sequential column on Day 205, EC: Continuous column on Day 205, T: Top, M: Middle, B: Bottom)

Figure 6-8: DGGE profiles of the fungal population in the sequentially and continuously fed columns on Day 160 and Day 205 of operation: (a) DGGE gel picture and (b) Digitized banding pattern.

Generally, more bacterial bands were observed under the nitrogen limited conditions than under the nitrogen rich conditions. In contrast, the DGGE results indicate that the fungal culture was spatially and temporally uniform in both columns and was not affected by the change in the nitrogen supply. These results are consistent with the DGGE findings discussed in Chapter 5 and indicate that even nitrogen limited conditions did not seem to affect the composition of the fungal community in the biofilters.

The similarity matrices developed from the DGGE results support the finding that the fungal population was significantly more stable with time and spatially more uniform than the bacterial population within the columns (See Tables 6-2 and 6-3). For examples, on Day 160 (N-limited conditions), the similarity of the fungal banding pattern was 0.89 (top-middle), 0.80 (top-bottom) and 0.91 (middle-bottom) in the CFC. The corresponding values in the SFC were 0.77, 0.73 and 0.86, respectively. Comparing biomass samples collected on Day 160 and Day 205 yielded Dice Index values of 0.80 (top), 0.57 (middle) and 0.92 (bottom) in the CFC and values of 1.00 (top), 0.80 (middle) and 0.88 (bottom) in the SFC.

In contrast, the composition of the bacterial population was greatly affected by the change in the nitrogen conditions within the column. For instance, the spatial similarity (Dice Index) of the bacterial banding pattern on Day 160 was 0.44 (top-middle), 0.00 (top-bottom) and 0.13 (middle-bottom) in the CFC. The temporal similarity of the bacterial banding pattern between Day 160 and Day 205 was 0.00 (top), 0.63(middle) and 0.00(bottom) in the SFC. Theoretically, a similarity value of S_D =0 indicates that the bacterial population on Day 160 was completely different than that present on Day 205. A complete turnover in the bacterial population is unlikely to have occurred over such a short period of time (i.e., 45 days). Since the similarity index was based only on the

presence or absence of DGGE bands, the DGGE results may have been biased during sample preparation (i.e., detaching biomass from the packing media), DNA extraction, or PCR amplification. Further discussions about possible sources of error in the DGGE technique are discussed below. Although the absolute value of the similarity value may be biased, the trends in the DGGE banding patterns are clear. That is, the bacterial population was much more affected by the limited nitrogen condition than was the fungal population. Since the VOC degradation patterns were also affected by the nitrogen conditions in the bioreactor, these results imply that the degradation of the less degradable VOCs (e.g., toluene and xylene) was more a function of the bacterial population in the bioreactor than the fungal population.

Table 6-2: Similarity matrix of the bacterial DGGE bands observed in the sequentially and continuously fed columns on Day 160 (N-limited) and Day 205 (N-rich) using the Dice index.

Dice (Czekanowski or Sorenson) Measure														
Case		I	LS			LC			ES			EC		
			T	M	B	T	M	B	T	M	B	T	M	B
I														
LS	T	.56												
	M	.59	**.92**											
	B	.54	**.55**	**.57**										
LC	T	.25	.50	.36	.30									
	M	.58	.60	.53	.71	**.44**								
	B	.15	.22	.25	.24	**.00**	**.13**							
ES	T	.00	*.00*	.00	.00	.00	.00	.00						
	M	.67	.70	*.63*	.57	.44	.69	.13	**.00**					
	B	.00	.00	.00	*.00*	.00	.00	.00	**.00**	**.00**				
EC	T	.15	.44	.50	.24	*.57*	.27	.00	.00	.27	.000			
	M	.35	.46	.50	.57	.18	*.63*	.25	.00	.42	.000	**.25**		
	B	.43	.40	.44	.22	.00	.25	*.40*	.00	.38	.000	**.00**	**.44**	

(I: Inoculum, LS: Sequential column on Day 160, LC: Continuous column on Day 160, ES: Sequential column on Day 205, EC: Continuous column on Day 205, T: Top, M: Middle, B: Bottom, Bold numbers indicate the similarity between bacterial populations present in different spatial locations within the columns. Italic numbers indicate the similarity of bacterial populations present in the same biofilter section on Day 160 and Day 205 of operation.)

Table 6-3: Similarity matrix of the fungal DGGE bands observed in the sequentially and continuously fed columns on Day 160 and Day 205 using the Dice index.

Dice (Czekanowski or Sorenson) Measure														
Case		I	LS			LC			ES			EC		
			T	M	B	T	M	B	T	M	B	T	M	B
I														
LS	T	.33												
	M	.22	**.77**											
	B	.29	**.73**	**.86**										
LC	T	.00	.89	.67	.60									
	M	.33	1.00	.77	.73	**.89**								
	B	.29	.91	.86	.83	**.80**	**.91**							
ES	T	.33	*1.00*	.77	.73	.89	1.00	.91						
	M	.25	.83	*.80*	.77	.73	.83	.77	**.83**					
	B	.40	.89	.67	*.80*	.75	.89	.80	**.89**	**.73**				
EC	T	.29	.91	.86	.83	*.80*	.91	.83	.91	.92	.80			
	M	.00	.57	.40	.50	.67	*.57*	.50	.57	.44	.67	**.50**		
	B	.25	.83	.80	.77	.73	.83	*.92*	.83	.86	.73	**.77**	**.44**	

(I: Inoculum, LS: Sequential column on Day 160, LC: Continuous column on Day 160, ES: Sequential column on Day 205, EC: Continuous column on Day 205, T: Top, M: Middle, B: Bottom, Bold numbers indicate the similarity between fungal populations present in different spatial locations within the columns. Italic numbers indicate the similarity of fungal populations present in the same biofilter section on Day 160 and Day 205 of operation.)

As is evident in the bacterial DGGE gel (Figure 6-7), fewer bacterial bands were detected in the nitrogen rich biomass samples than in the nitrogen limited biomass samples. Although there are several possible explanations for this result, one must consider the limitations of the DGGE technique. That is, the PCR-DGGE technique is semi-quantitative at best. First of all, PCR itself is an important source of errors and biases in molecular studies of environmental samples (Muyzer and Smalla, 1998). Second, differences in the efficiency of DNA extraction from different cell types are likely to exist. The 16S rRNA copy number per cell is known to vary, and the kinetics of PCR amplification of the different molecules present in samples containing a diversity of bacteria may not be uniform (Bruggemann *et al.*, 2000; Wantanabe *et al.*, 2001).

Nonetheless, PCR amplification of DNA and DGGE analysis of the products has provided a useful means to directly characterize many bacterial populations within samples. In this study, more diverse bacterial bands were observed in the bioreactors under nitrogen limited conditions than under nitrogen rich conditions. This result is rather contrary to the hypothesis that microorganisms that are adapted to high nitrogen concentrations will be the most significantly affected by nutrient limited conditions and the population of these microorganisms will thus diminish. However, as explained below, it is suspected that only the dominant microbial species may have been detected due to inherent biases that are possible when extracting DNA from the biofilm samples and subsequently amplifying it using PCR.

Under nitrogen rich conditions, a few faster growing organisms may dominate the culture and be selectively amplified in the DNA extraction and PCR steps. As a result, only a few bands representative of those organisms that grow fastest under nitrogen rich conditions would appear on the gels. However, under nitrogen limited conditions, the N-rich competitive organisms would not be able to compete with the N-limited competitive organisms. Since N-rich organisms would no longer dominate the culture, it is more likely to extract and amplify DNA from the variety of species present.

6.3 SUMMARY

The results in this chapter indicate that the biodegradation of the aromatic components of the paint mixture seemed to be the most sensitive to the nitrogen level present in the biofilm. In contrast to the rapid breakthrough observed for the aromatic hydrocarbons, MPK breakthrough did not occur for two weeks after the inorganic nitrogen concentration in the column was completely depleted and NBA and EEP were completely degraded throughout the entire nitrogen limited period. The microorganisms

were likely utilizing recycled organic nitrogen during this period. The order of breakthrough of each of the paint VOCs under nitrogen limited conditions was the exactly reverse of the order in which the VOCs were found to be degraded in the column during the start-up period. The DGGE analyses indicate that the nitrogen availability in the column significantly affected the bacterial community. Fewer bacterial DGGE bands were observed under nitrogen rich conditions than under nitrogen limited conditions. It is likely that a few fast growing organisms dominate the community under nitrogen rich conditions and were amplified in the DNA extraction and PCR steps of the DGGE analysis. However, the fungal population was much more stable with time and spatially more uniform than the bacterial population in the biofilter regardless of nitrogen availability.

Chapter 7 Transient Loading Response and VOC Elimination Capacity

Under steady feed conditions, vapor phase bioreactors have proven to be an effective technology for removing odors and volatile organic compounds from waste gas streams (van Groenestijin and Hesselink 1994; Wani *et al*., 1997). However, when these systems are subjected to dynamic feed conditions or shutdown and restart situations, reliable performance is more difficult to maintain (van Groenestijin 1994; Martin 1996; Wani 1997; Tang 1996;.Choi 1998; Park 2001) Because transient feeding conditions are very common in field applications, biofilter performance needs to be stable even under frequent shutdown/restart events. Such intermittent operation is typical of paint spray booths and many other industrial processes. Thus, it is important for biofilters for paint booth applications to be able to maintain reliable performance under transient loading conditions. Otherwise, another backup system would be required to consistently meet stringent emission regulations.

In this phase of the research, the reliability of biofilter performance was investigated under transient feeding conditions. Loss of biomass activity is expected following a shutdown period where the normal VOC feed to the bioreactor is interrupted (Wani 1997; Martin 1996; van Groenestijin 1994). One strategy that has been investigated to improve the response of biofilters to periods of non-use is to provide a slip feed system during the shutdown period. In a previous study with a single VOC feed, a slip feed system was found to maintain the pollutant degrading capacity of the biomass during the shutdown and reduced the re-acclimation time required following 3 and 7 day shutdown periods by as much as 70 % (Park 2001). The response of a biofilter treating a paint mixture to interruptions in the VOC feed is unknown. Thus, the transient load

experiments described below provide the basis for assessing the feasibility of applying vapor-phase bioreactors to treat intermittent emissions from paint spray booths and similar facilities. Another key factor that must be considered when evaluating biofiltration technology for a paint spray booth application is the size of the biofilter unit and its pollutant elimination capacity. Biofilter performance was assessed over a range of empty bed contact times and inlet VOC concentrations. Based on these results, the VOC elimination capacity of the biofilter as function of VOC loading rate was established.

7.1 EXPERIMENTAL METHODS

The same SFC and CFC biofilters used in the previous experiments described in Chapters 5, 6 and 7 was utilized for the transient feed experiments. The biofilters had achieved steady state removal of the surrogate paint VOCs for a period of 205 days before they were subjected to a series of transient loading experiments. In these experiments, the influent VOC supply was intermittently turned off and then back on to assess the transient response of the biofilter following periods of no loading. In these transient loading experiments, each biofilter was fed VOC-contaminated air for 6 hours per day, 5 days per week, to simulate loading conditions where paint off-gases are generated only during a fraction of each day and no contaminants are generated during weekend shutdown periods. During the time intervals when no VOCs were provide to the biofilters, each biofilter continued to receive humidified air at the same flow rate as it did during periods in which VOCs were supplied. In addition to the 18-hour shutdown each day, three different shutdown periods (i.e., weekend, an extended weekend, and a long-term shutdown) were investigated during this experiment. VOC removal efficiencies

were monitored over time after each shutdown period to see how quickly the biofilters could recover their pollutant degrading capacity. In addition, degradation profiles of the paint mixtures along the column were monitored to determine how transient loading conditions affected the VOC degradation patterns. At the end of biofilter experiments, the elimination capacity of the biofilter was measured for various inlet VOC concentrations and empty bed contact times. The elimination capacity tests were conducted over six days. One set of experiments were conducted at an EBCT of 30 sec and inlet VOC concentrations of 10, 100 and 200 ppm_v and a second set of experiments were conducted at an EBCT of 15 sec and total inlet VOC concentrations of 10, 100 and 300 ppm_v. At each VOC concentration, ratio of each VOC in the paint mixture (by volume) was maintained at the same ratio as presented in Table 3-2.

7.2 RESULTS AND DISCUSSION

7.2.1 Overall Bioreactor Performance during Transient Loading

The results of the transient loading experiments are summarized in Figure 7.1. Each data point in Figure 7-1 is the average VOC removal efficiency observed over the 6 hours per day when the biofilters were operating. The first day of the transient experiments with the biofilters is designated as Day 1 in Figure 8-1. Prior to these experiments, the SFC and CFC systems had been continuously operating for more than 200 days as described previously in Chapters 4 and 6.

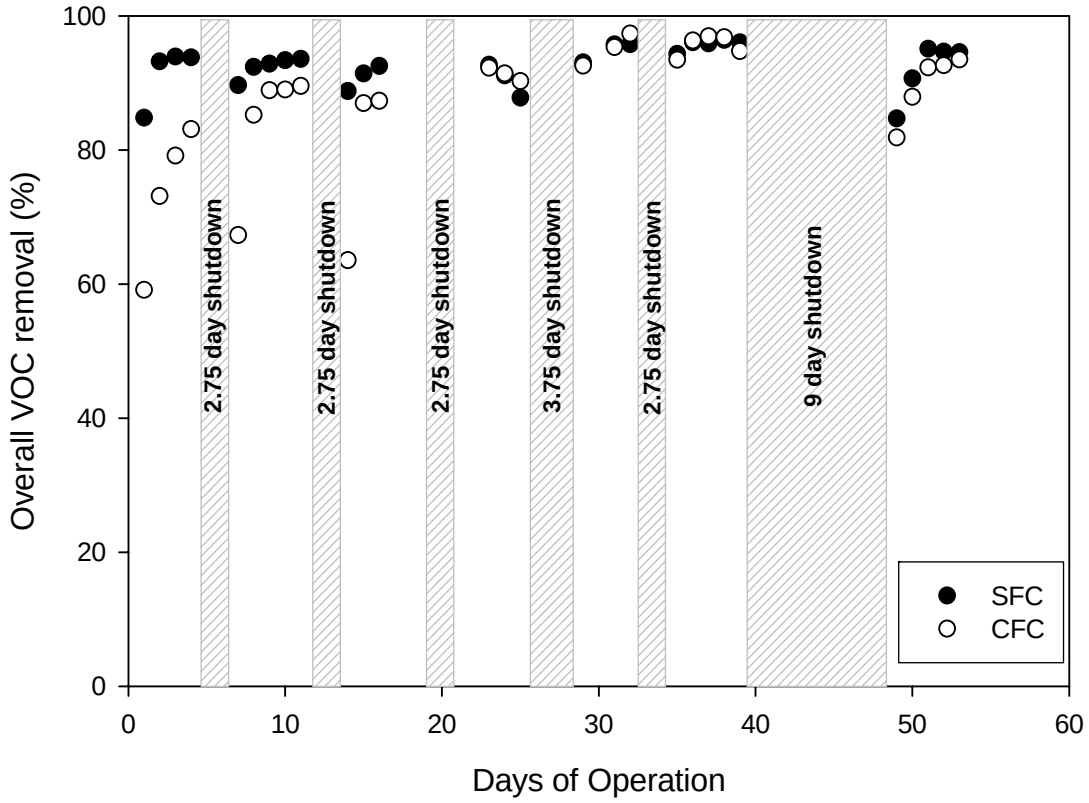


Figure 7-1: VOC removal efficiencies observed during the transient loading experiments.

The results presented in Figure 7-1 indicate that the SFC biofilter was more resilient to transient loading conditions than the CFC biofilter at the beginning of the transient experiments. The SFC biofilter achieved higher MPK and toluene removals and significantly greater xylene degradation than the CFC biofilter. The higher xylene removal in the SFC was not surprising given that the xylene removal in this reactor was greater than that in the CFC during the continuous feed experiments. It is interesting to note, however, that the SFC recovered MPK and toluene removal much more rapidly following each shutdown period than the CFC even though both the SFC and CFC columns had similar MPK and toluene removal efficiencies prior to the beginning of the

transient feed experiments. This result suggests that the sequential feeding strategy enriched the microbial population capable of degrading MPK and toluene in the SFC biofilter and provided the SFC with an initial advantage during the transient feed experiments. However, the differences in the VOC removal efficiencies between the two bioreactor columns became smaller as both columns acclimated to operating under transient loading conditions.

Another interesting result from the transient feed experiments is that the CFC biofilter achieved higher VOC removal under transient loading conditions than it did under continuous loading conditions. One possible explanation for this result is that one or more chemical in the paint mixture was not completely degraded during continuous feeding conditions. The buildup of intermediate byproducts may have inhibited the degradation of the other volatile constituents in the paint mixture under continuous loading conditions. However, under transient loading conditions, intermediate biodegradation products that are produced during the contaminant feed period have an opportunity to be degraded during the feed shut-down period when only air is provided to the column. The results of each shutdown experiment are described in more detail below.

7.2.2 A Weekend Shutdown Experiment (2.75 days)

The VOC loading to each bioreactor was discontinued for a period of 2.75 days in order to simulate a weekend shutdown event. Following the shutdown period, the VOCs were provided to the biofilter for a period of 6 hours per day. Figure 7-2 shows the VOC removal efficiencies observed during the first 6 hours following the first weekend shutdown experiment (on Day 8).

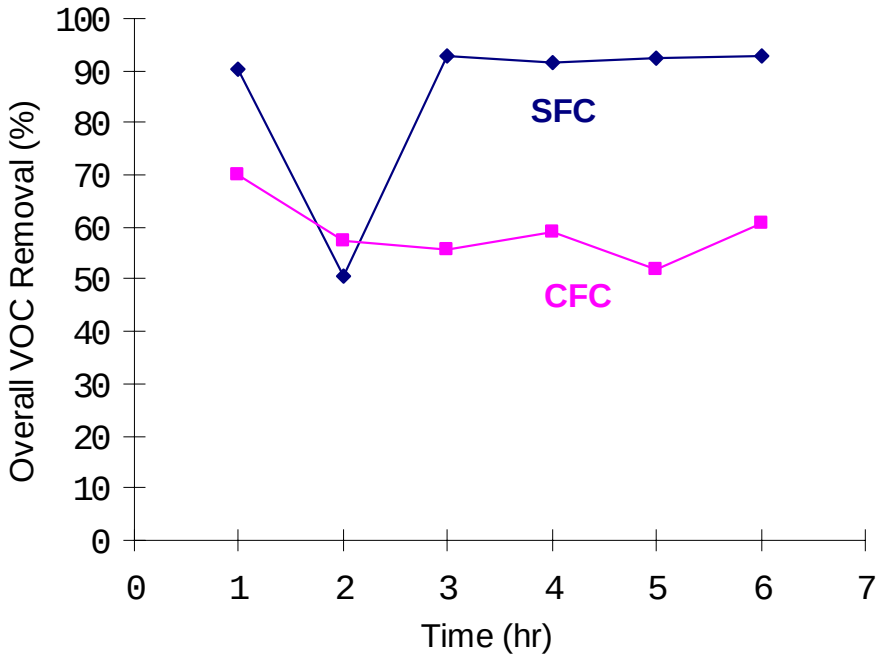


Figure 7-2: VOC removal efficiencies observed on the first day following a weekend shutdown (2.75 days) (Note: The six-hour operation on Day 8)

As can be seen in Figure 7-2, the SFC achieved 90% removal of the paint mixture one hour following restart of the VOC feed but the removal efficiency dropped to 50% during the second hour. The initially high removal efficiency may have resulted from absorption of MPK during the first hour of operation. After MPK equilibrated with liquid in the column, greater MPK breakthrough was observed over the next hour until the MPK biodegradation rate in the column recovered. An overall removal efficiency of 90% was achieved within 3 hours of restart, similar to results reported by Moe and Qi (2004). In contrast, the recovery of the CFC was not nearly as rapid, and the CFC achieved only 60% VOC removal in the six hours following column restart.

After each bioreactor had been supplied with a VOC feed for 6 hrs, no organic chemical feed was provided to either column for the next 18 hrs. The biofilter was then provided with the surrogate paint VOC mixture again for six hours. The response of the biofilter is summarized in Figure 7-3.

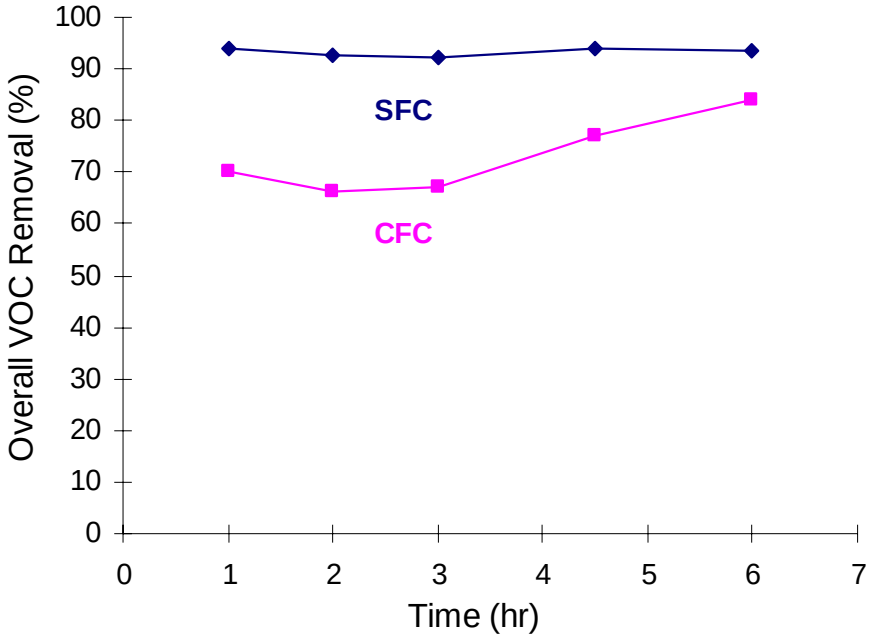


Figure 7-3: VOC removal efficiencies observed on the second day following a weekend shutdown (2.75 days) (Note: The six-hour operation on Day 9)

The SFC sustained greater than 90% removal of the paint mixture over the entire six hour VOC feed period on the second day following the weekend shutdown experiment. The CFC biofilter also recovered its pollutant degrading capacity, though more slowly, eventually achieving greater than 80 % removal of the paint mixture by the end of the second day.

On day 14, following the second weekend shutdown, the CFC biofilter still slowly recovered compared to the SFC biofilter. Following the third weekend shutdown period, unfortunately, the VOC removal efficiency in the biofilters could not be measured for two days (On Day 21 and Day 22). However, during the rest of the week (Day 23, 24, and 25), both SFC and CFC biofilters could achieve similar VOC removal.

7.2.3 An Extended Weekend Shutdown Experiment (3.75 days)

After the biofilters had been operated with an intermittent chemical feed for 25 days (i.e., 6 hrs per day, 5 days per week), the biofilters were subjected to an extended 3.75 day shutdown period. Since both biofilters had been operating under transient loading conditions for the 25 days prior to this shutdown experiment, the columns were more resilient to shutdown/restart events (Figure 7-4). Both the SFC and CFC biofilters removed greater than 90% of the paint mixture immediately after column restart, and this high removal efficiency was maintained throughout the day when the chemical feed was re-supplied following the shutdown.

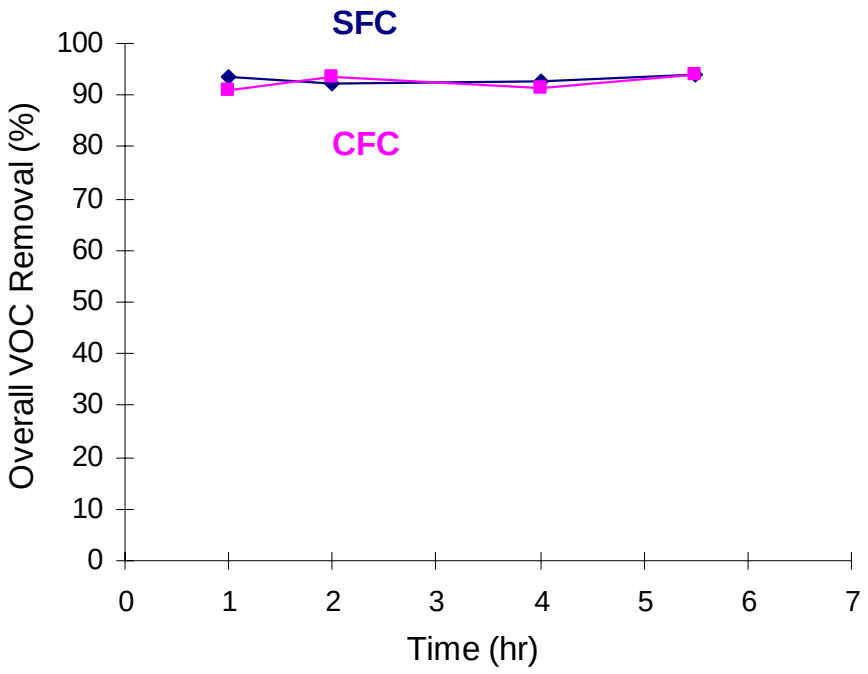


Figure 7-4: VOC removal efficiencies over the first six-hour operation following an extended weekend shutdown (3.75 days) on Day 29

7.2.4 A Long-Term Shutdown Experiment (9 days)

The response of the biofilter to a longer, nine-day shutdown period was also investigated. Such a shutdown period may occur in full-scale applications as a result of system maintenance or changes in painting schedules. The recovery of each biofilter following the nine-day shutdown experiment is summarized in Figure 7-5.

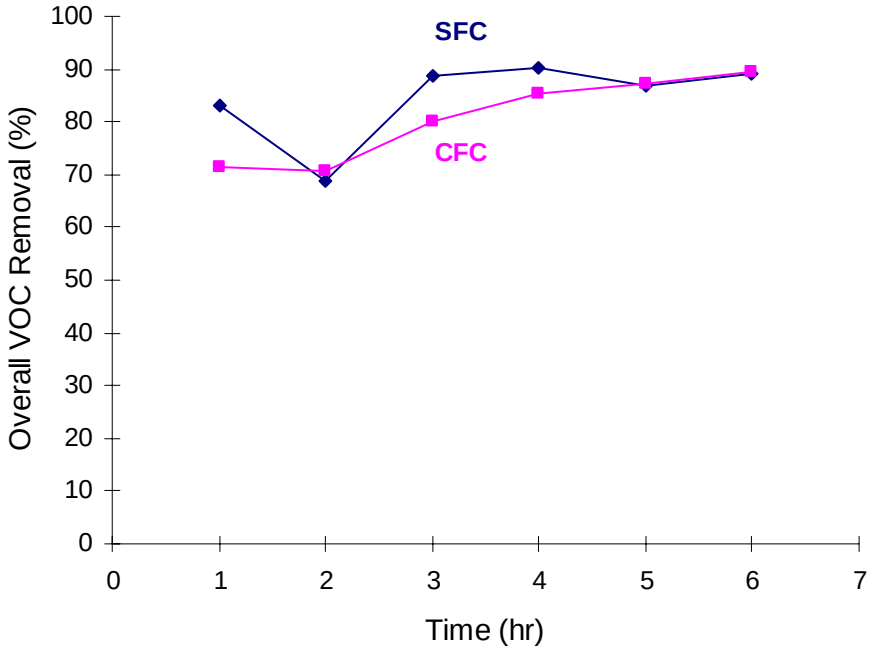


Figure 7-5: VOC removal efficiencies over the first six-hour operation following long-term shutdown (9 days) on Day 49

Even though the initial VOC removal efficiency was slightly lower (85% and 70% in the SFC and CFCs, respectively) in the long-term shutdown experiment than in the shorter shutdown experiments, both biofilters recovered their pollutant degrading capacity relatively quickly. It is interesting to note that the initial response on day 49 following the 9-day shutdown period was better than that observed on day 7 following the 3-day shutdown period. These results suggest that microbial populations in biofilters can adapt to transient loading conditions and that the initial response of the biofilters following shutdown periods can improve over time.

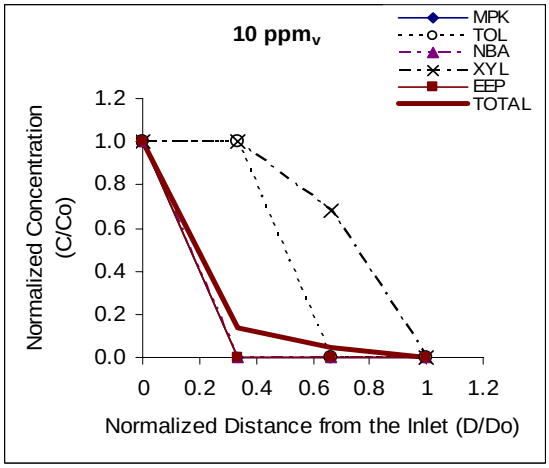
7.2.5 Empty Bed Contact Time and Elimination Capacity (EC) Tests

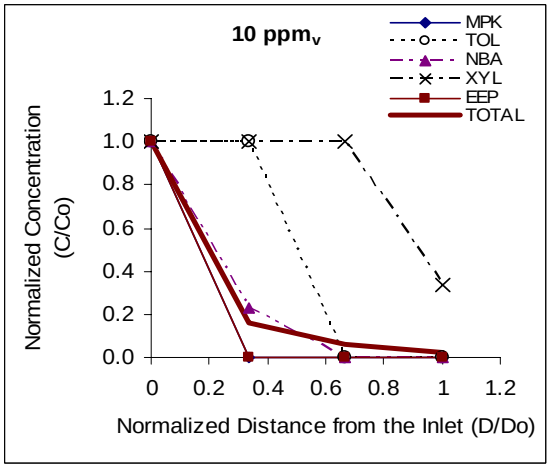
To assess performance of the biofilter over a range of operating conditions, a series of empty bed contact time (EBCT) experiments were conducted at several different pollutant concentrations. Due to the mechanical limits of the air flow system available to supply the laboratory-scale biofilter, the shortest EBCT tested in this experiment was 15 seconds. Figure 7-6 summarizes the VOC removal profiles across each bioreactor column at each experimental loading condition tested.

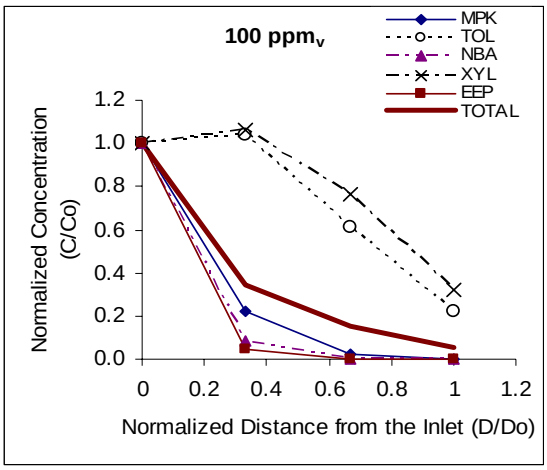
At a 30 sec EBCT, the CFC achieved 100 % overall VOC removal of a 10 ppm_v feed, 95% removal of a 100 ppm_v feed and 83% removal of a 200 ppm_v feed. At a 15 sec EBCT, the CFC biofilter removed 98% of a 10 ppm_v VOC feed. When the inlet concentration was increased to 100 ppm_v at a 15 sec EBCT (which yields an inlet loading similar to that when 200 ppm_v was provided at a 30 sec EBCT), the overall VOC removal was approximately 80%. When the biofilter was supplied with 300 ppm_v of VOC at a 15 sec EBCT, approximately 70 % of the inlet VOCs were removed. In terms of overall VOC removal efficiency, these two different EBCT experiments yielded similar VOC removal efficiencies at similar inlet VOC loadings. However, higher inlet concentrations yielded a more exponential degradation pattern due to the increase in the concentration gradient force which improves pollutant removal in the biofilters. The VOC removal observed in the SFC was quite similar to that observed in the CFC, indicating that at this point in the operation of the biofilters (e.g., after 270 days of operation), neither biofilter provided any advantage with respect to VOC removal efficiency at shorter EBCTs.

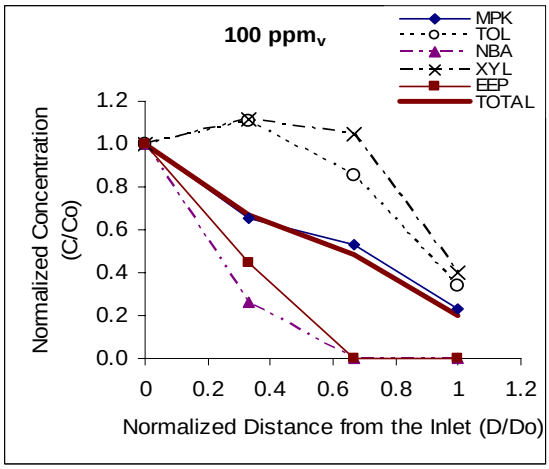
I. Continuouslv Fed Column

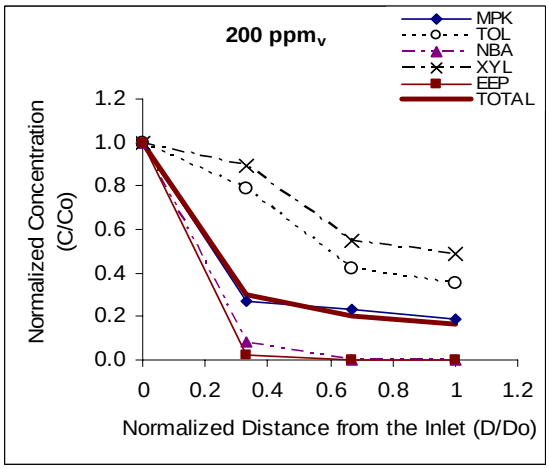
(a) EBCT: 30 sec **(b) EBCT: 15 sec**

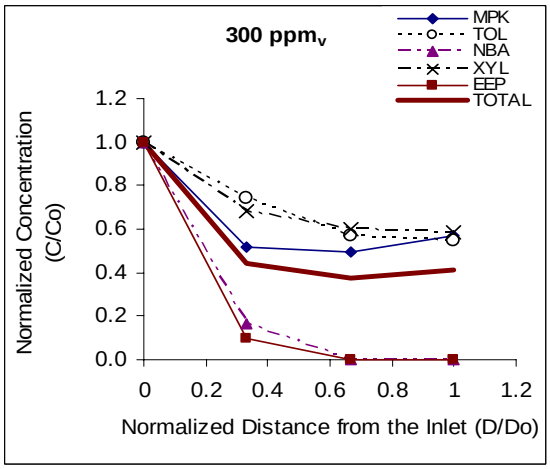












II. Sequentially Fed Column

(a) EBCT: 30 sec **(b) EBCT: 15 sec**

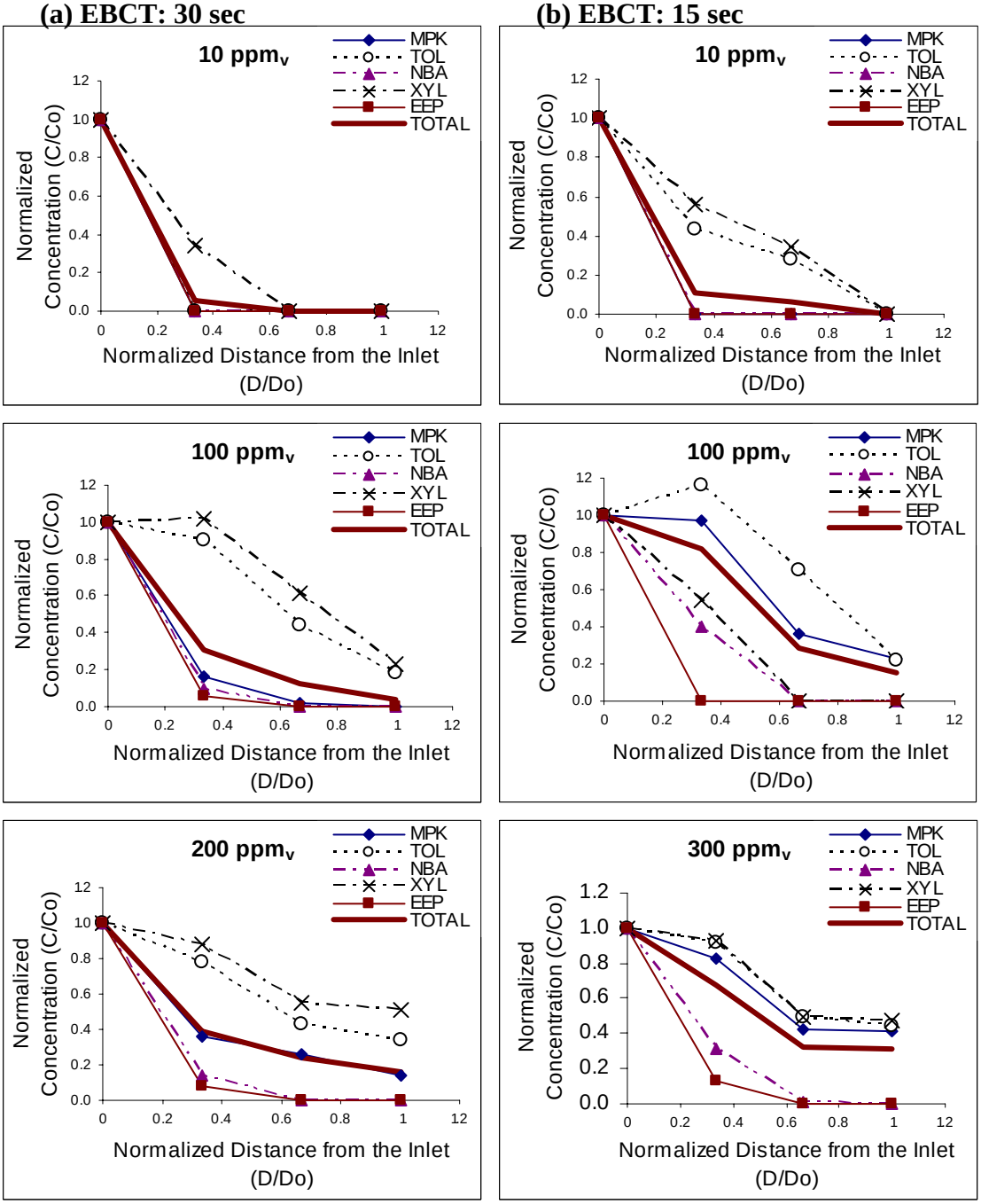


Figure 7-6: VOC removal profiles in the continuously fed column (I) and the sequentially fed column (II) at a 30 sec and 15 sec EBCT. The total inlet VOC concentration is indicated at the top of each figure.

The elimination capacity of each biofilter as a function VOC load is summarized in Figure 7-7. The data presented in this figure includes all the data obtained at the three different EBCTs (1 minutes, 30 sec, and 15 sec) and the range of VOC concentrations examined in these experiments.

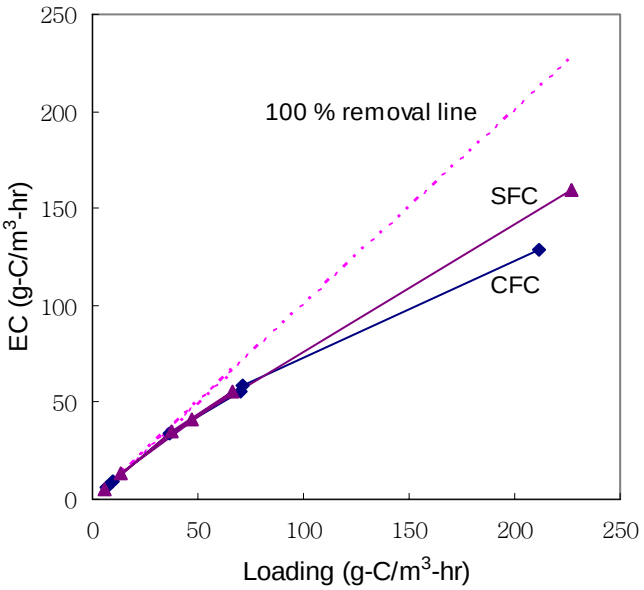


Figure 7-7: Elimination capacity of the continuously fed and sequentially fed columns as a function of VOC loading rate at a 15 sec and 30 sec EBCT.

No significant differences in the EC curves were observed for the SFC and the CFC for loads up to 70 g-C/m^3-hr. These results indicate that the foam biofilter can successfully treat paint VOC mixtures within the operating range tested in this experiment. Biofilter performance was acceptable when treating relatively high gas flow rates (EBCT 15 sec) for VOC concentrations ranging from 10 to 100 ppmv. In addition, throughout the entire operating period of the lab-scale biofilter experiment, the pressure drop across the packed column was negligible (less than 0.2 in H_2O).

7.3 SUMMARY

The SFC and CFC biofilters which had been continuously operated for more than 200 days were quite resilient to transient loading conditions and tolerated shutdown periods up to 9 days. In contrast to other studies, no additional supply of carbon was required during the off period to maintain the pollutant degradation capacity. In fact, the performance of the SFC and CFC bioreactors improved as they became acclimated to operating under transient load conditions. Also, overall performance of the biofilter system used in this study was found to be acceptable for EBCTs as low as 15 seconds and VOC concentrations ranging from 10 to 100 ppm_v.

Chapter 8 Pilot Scale Evaluation

Actual paint spray booth operations generate waste gases containing VOC mixtures that vary in concentration and composition over short time periods. Paint booths also operate intermittently and typically shut down during evenings and weekends. Paint booth emissions thus pose a real challenge to biofilters since biological treatment systems often have more difficulty treating intermittent waste streams containing complex mixtures of pollutants. Generally, more complex substrate interactions and microbial competition is expected in field bioreactors since the substrate composition is more complicated in actual paint mixtures. The laboratory-scale studies discussed in the previous chapters laid the foundation for determining the capabilities of biofiltration technology to treat paint spray booth emissions. Nevertheless, a series of pilot-scale experiments were necessary to assess the ability of biofiltration technology to treat actual paint emissions under the challenging conditions expected in the field. To this end, the performance of a pilot-scale biofilter treating actual paint emissions on an intermittent basis was investigated. Scale-up issues such as bioreactor start up procedures as well as slip feed design are also addressed in this chapter.

8.1 EXPERIMENTAL METHODS

8.1.1 Pilot Scale Bioreactor Design

A pilot-scale bioreactor was constructed for this Task as illustrated in Figures 8-1 and 8-2. The basic design of the pilot-scale bioreactor was based on the biofilter system used in the previous laboratory-scale study. The pilot-scale bioreactor consisted of a stainless steel column with an inner diameter of 0.61 m and a total height of 3.25 m. The

column was divided into three sections. The bottom section served as a nutrient sump and housed the inlet gas distribution system which consisted of a horizontal perforated pipe. The perforations were spaced approximately 2 cm apart and were pointed downward to force the inlet gas stream to reverse direction (and mix) prior to entering the packing material. The top section housed the nutrient spray distribution system, and the middle section was packed with 444 L of polyurethane foam cubes (5 cm per side). To humidify the air entering the biofilter, a humidifier (inner diameter of 0.15m, total height of 1.97m) was constructed. The humidifier was packed with 5/8" pall rings (total packed volume of 22.2 L) through which tap water was continuously recirculated at a rate of 2 gal/min.

A scaled-down paint booth was constructed to feed actual paint spray emissions to the biofilter (see Figure 8-3). The paint booth consisted of a paint spray gun housed in a duct through which air continuously flowed at rate of 24.5 scfm. The VOC-laden exhaust stream exiting from the paint booth was pre-filtered (Chemco, Inc., HI Solids 2 Pad) to remove particulate matter and then ducted to the humidifier unit located immediately upstream of the biofilter. The quantity and rate of paint sprayed through the atomizing paint nozzle was controlled by regulating the pressure of a paint kettle that contained a mixture of paint and paint thinner. The frequency of the paint spray was controlled by a switch that was programmed to supply paint to the paint gun at 10-second intervals when the paint booth was "operating".

Figure 8-1: Photograph of the pilot-scale bioreactor.

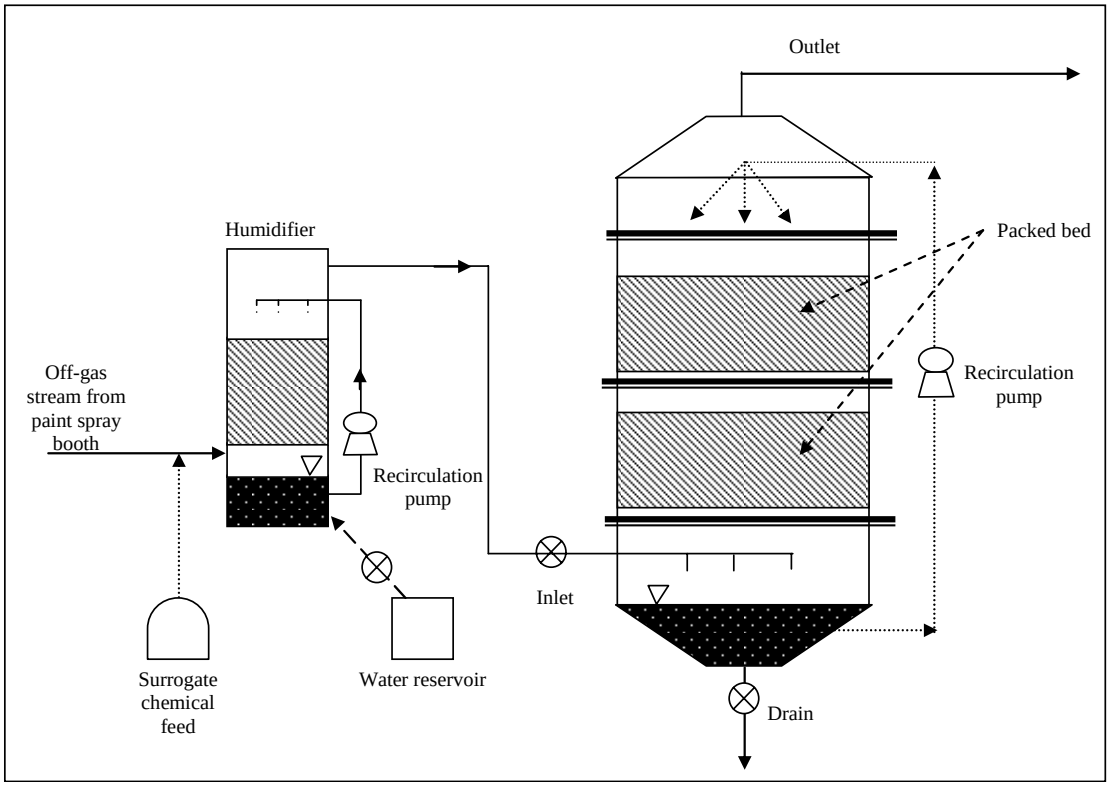


Figure 8-2: Schematic diagram of the pilot-scale bioreactor.

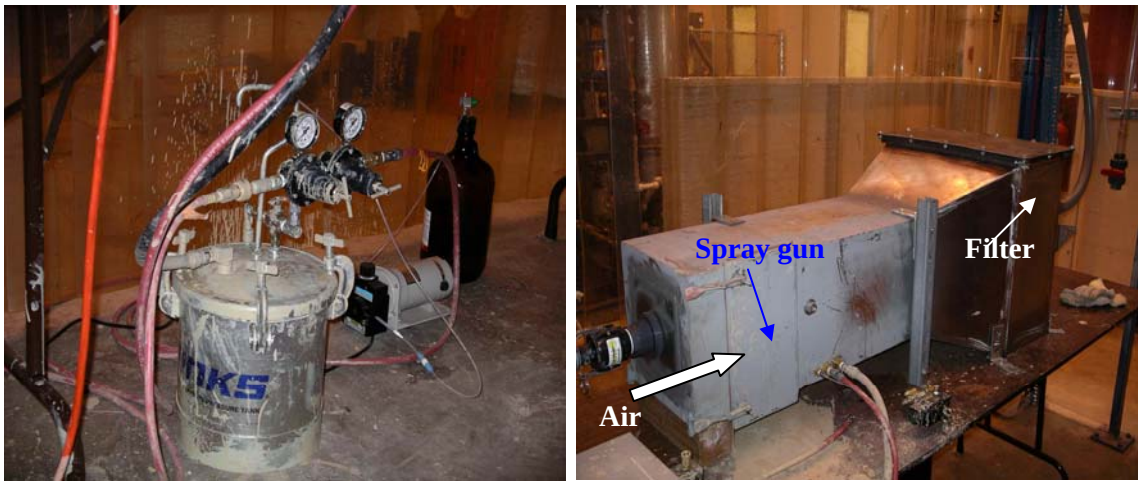


Figure 8-3: Paint booth system used in pilot-scale tests (Left: paint kettle; Right: paint spray duct)

8.1.2 Paint/Thinner Mixture

Several factors were considered when selecting the types of paint used in the pilot-scale study. First, the paint selected had to have an adequate "pot-life"- the time period that the paint could be sprayed from the paint kettle before it hardened and could not be sprayed further. A pot life of at least 6 hours was necessary in order to feasibly run the pilot test without excessive changes of the paint kettle and cleaning of the paint gun. Second, the paint selected needed to contain the VOCs most commonly found in paint VOC mixtures at maintenance operations. Discussions with painting personnel at Ft. Hood, Texas indicated that the tan paint selected for use in this study is a very common paint (Ft. Hood, 2003). Furthermore, inspection of Material Safety Data Sheet (MSDS) information and emission inventory data from Ft. Hood and Anniston Army Depot as well as paint booth emissions data obtained from Tyndall AFB indicated that

the major VOCs emitted from paint booths at these facilities are found in the paint selected for study. The third consideration was that the composition of the paint should be one likely to prove challenging to biofiltration technology in order to assure that adequate performance could be achieved even with unfavorable paint formulations.

The paint selected for testing contained 3.5 lbs VOCs/gallon which is higher than the 1.5 lbs VOCs/gallon that can be found in low VOC paints. Also, the paint thinner selected contained both xylene and toluene, components which were found to be the most recalcitrant to biodegradation in the previous biofilter studies. The paint was mixed with this thinner in a 3:1 ratio to ensure that aromatic hydrocarbon emissions were included in sufficiently high concentrations to challenge the capabilities of the pilot-scale biofilter and also to prevent the scaled-down paint gun from clogging. Table 8-1 summarizes the physical and chemical properties of the paint and thinner used in the pilot-scale experiments. The VOC composition of the paint and thinner are presented separately in Table 8-2.

Table 8-1: Physical and chemical properties of the paint and thinner used in the pilot-scale study.[(1)]

Property	**Paint**	**Thinner**
Evaporation rate (n-Butyl acetate =1)	1.6	5.6
Vapor pressure (mm Hg)	15	74
Vapor density (g/L)	4	4.6
VOC (lb/gal)	3.494	7.424
VOC (g/L)	418.68	889.61
Weight per gallon (lb/gal)	10.1491	7.4329
Specific gravity	1.219	0.892

(1) MSDS from Hentzen Coating Inc., 2004

Table 8-2: Properties of the VOCs found in the paint and thinner used in the pilot-scale study.[1]

Paint (Tan 686A zenthane, Mil-C-53039A)				
Compound	CAS #	MW	Density(g/mL)	%(by weight)
methyl isoamyl ketone (MIAK)	110-12-3	114.187	0.814	25.325
methyl amyl ketone (MAK)	110-43-0	114.187	0.815	1.164
methyl isobutyl ketone (MIBK)	108-10-1	100.16	0.7978	4.461
butyl acetate (BA)	123-86-4	116.16	0.882	1.261
Thinner (Type I, MIL-T-81772B)				
Compound	CAS #	MW	Density(g/mL)	%(by weight)
Toluene	108-88-3	92.15	0.8996	11.787
Xylene	13330-20-7	318.501	0.862	6.774
Ethyl benzene (EB)	100-41-4	106.167	0.867	0.986
Butyl acetate (NBA)	123-86-4	116.16	0.882	9.9
methoxypropanol acetate (MA)	108-65-6	132.159	0.969	43.427
methyl ethyl ketone (MEK)	78-93-3	72.1066	0.805	27.115

(1) MSDS from Hentzen Coating Inc., 2004

8.1.3 Analytical Methods

The same analytical methods described in Chapter 3 were used in this phase of the research except for the VOC gas measurements. The details of the gas chromatography method used for VOC measurement are as follows.

VOC Gas Measurements

Gas samples were periodically collected and analyzed in the HP 6890 Gas Chromatography (GC) as previously described in Chapter 3. However, there was a slight change of gas flow rate and temperature setting in this GC method. As a carrier gas, He was set at a flow rate of 1.0 mL/min. The make-up gas flow to the detector consisted of He (25 mL/min), H_2 (45 mL/min) and zero degrade air (300 mL/min). The column temperature started at 60 ^{o}C for 1 min and increased at the rate of 2 ^{o}C/min to 75 ^{o}C and hold 70 ^{o}C for 2 min.

8.1.4 Inoculation

To establish an effective biofilm within the pilot-scale biofilter during startup, the bioreactor was initially inoculated with microbial cultures capable of degrading the key constituents found in paint VOC emissions. Because of the VOC composition of the paint tested, one microbial culture (initially obtained from another bioreactor treating a mixture of paint VOCs) was grown with 100 mg-C/L methyl isoamyl ketone(MIAK) as the sole carbon and energy source. As shown in Table 8-2, MIAK represents the ketone found in the highest fraction in the paint used in the pilot study. Also, because the laboratory-scale studies indicated that the aromatic compounds are the most difficult compounds to degrade in the paint VOC mixture, a separate aromatic degrading culture was developed using leachate derived from a different laboratory-scale bioreactor treating benzene, toluene, ethyl benzene and o, m, and p-xylenes (BTEX). The aromatic-degrading culture was grown up in the presence of 100 mg-C/L each of BTEX as the carbon and energy source. After the MIAK-degrading culture and the aromatic-degrading cultures could degrade repeated injections of the VOCs, these two cultures were mixed together and transferred to the sump of the pilot-scale bioreactor. The nutrient medium for the inoculation culture in this task was identical to the one as described in Chapters 3 (see Table 3-3). The 40 L of inoculating culture was recirculated through the bioreactor for 12 hrs prior to starting waste gas feed to the bioreactor.

8.1.5 Bioreactor Start-up Period

The pilot-scale bioreactor was operated as a biofilter as described earlier in Chapter 4. The total gas flow rate through the system was maintained at 24.5 scfm which corresponds to an empty bed contact time of 38 seconds in the biotrickling filter

column. A nutrient solution (10.1 g/L KNO_3, 1.36 g/L KH_2PO_4, 0.71 g/L Na_2HPO_4, 0.66 g/L $(NH_4)_2HPO_4$) was intermittently recirculated through the bioreactor at a rate of 8 gal/min using the nutrient spray distribution system located at the top of the foam packing material. The nutrient solution was recirculated through the foam packing for intervals of 30 to 40 minutes at a frequency of two to three times per day. Once per week, half of the nutrient solution in the column was replaced with fresh nutrient solution. The intermittent biotrickling filter was initially fed a surrogate VOC mixture (Table 8-3) continuously for approximately one month to facilitate biomass establishment on the biofilter's foam packing medium prior to treating the actual paint off-gas stream.

Table 8-3: Composition of the surrogate VOC feed during the start-up period.

Day	Average VOC concentration, ppm_v			
	toluene	n-butyl acetate (NBA)	p-xylene	Total VOC
0 - 20	39 (18-73)*	26 (17-44)	41 (15-80)	106
21 - 25	58 (46-66)		81 (66-94)	140
26 - 34	112 (66-155)			112

* The numbers in parenthesis indicate the range of VOC concentration that measured in the inlet.

The painting experiments began on Day 36 of operation and continued for 38 days. During the painting experiments, the biofilter was provided a waste gas stream from the paint spray booth for 6 hours per day, 5 days per week. During the time interval (18 hours per day) when no VOCs were supplied to the biofilter, the biofilter received only humidified air at the same air flow rate (off period).

The VOC composition of the inlet and outlet gas stream into the pilot bioreactor was monitored by gas chromatography as described earlier in Section 8.2.3. Seven major VOC components of the paint waste gas stream were monitored throughout the experiments. Other trace components in the waste gas stream were found to be present

at negligible quantities. In order to confirm the VOC composition, a few gas samples were collected in Suma canisters and sent to a commercial lab (EMSL Analytical, Westmont, NJ) for analysis by Gas Chromatography/Mass Spectrometry (GC/MS). Results of these analyses indicate that the composition of the waste gas as determined by GC/FID was consistent with that observed by GC/MS analysis and reported in the MSDSs for the paint and thinner products used in the experiments.

8.2 RESULTS AND DISCUSSION

A pilot-scale biofilter was set up and operated for 72 days. Throughout the entire operating period of the pilot-scale biofilter experiment, the pressure drop across the packed column was negligible (less than 0.5 in H_2O). For the initial 34 days, surrogate paint mixture was continuously supplied to the biofilter to facilitate the biomass establishment. Then, three phase of paint spraying experiments were conducted for next 38 days. The results are presented as follow:

8.2.1 Bioreactor Startup

As observed in the laboratory-scale biofilters treating a similar surrogate paint mixture, complete removal of n-butyl acetate and poor removal of the aromatic compounds was observed during the initial 34 day period of bioreactor operation. Figures 8-4, 8-5, and 8-6 summarize the removal efficiency and elimination capacity observed during this initial period for toluene, p-xylene, and total VOCs, respectively. Due to difficulties in controlling the VOC-feed rate, the VOC concentration in the inlet waste gas stream varied considerably (see Table 8-3). As a result, the removal efficiency and elimination capacity varied depending on the inlet concentration during this start-up period.

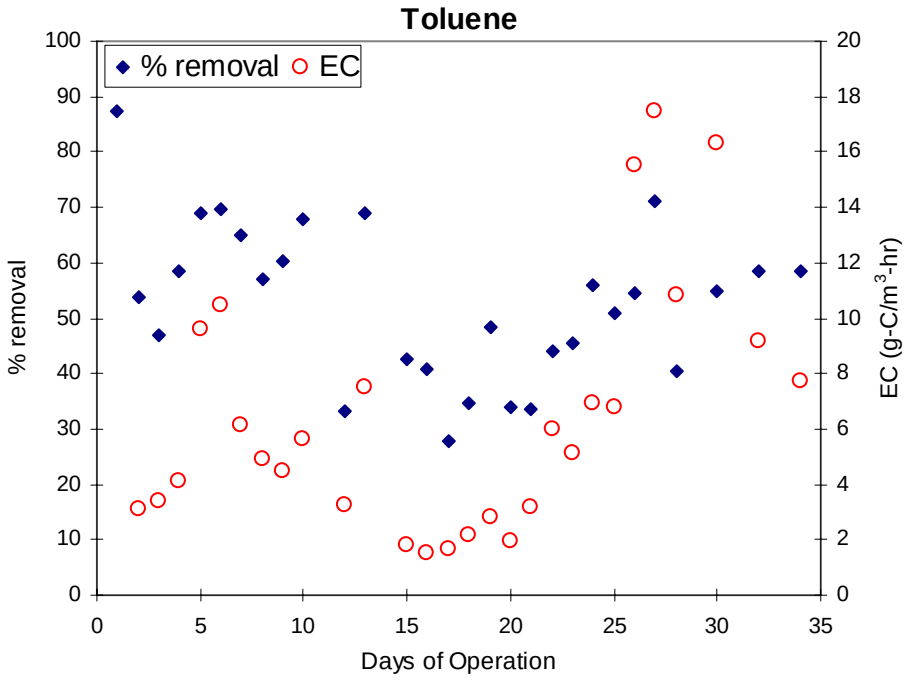


Figure 8-4: Toluene removal efficiency and elimination capacity during the start-up period.

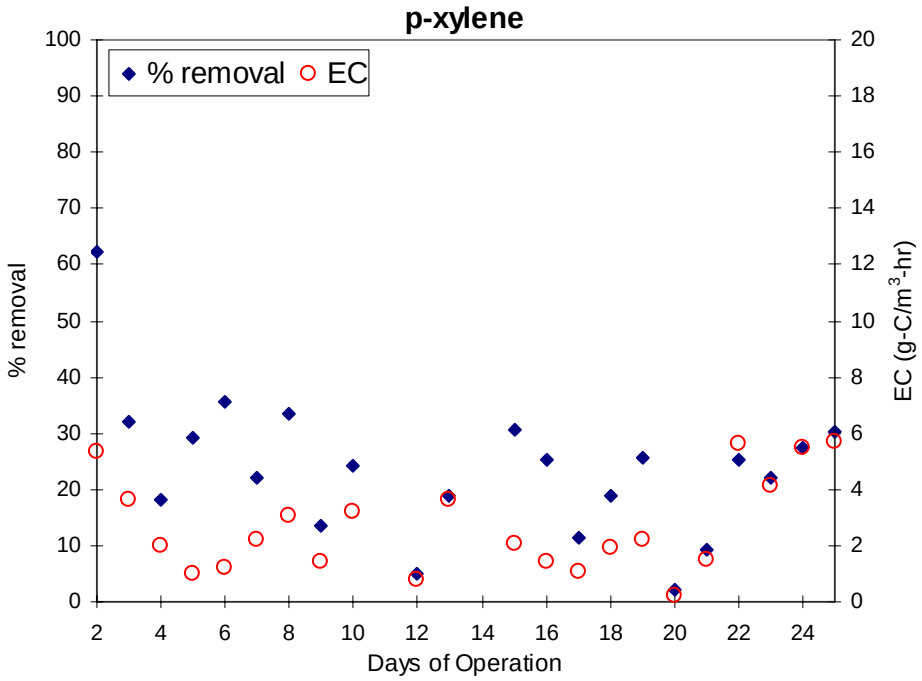


Figure 8-5: p-xylene removal efficiency and elimination capacity during the start-up period.

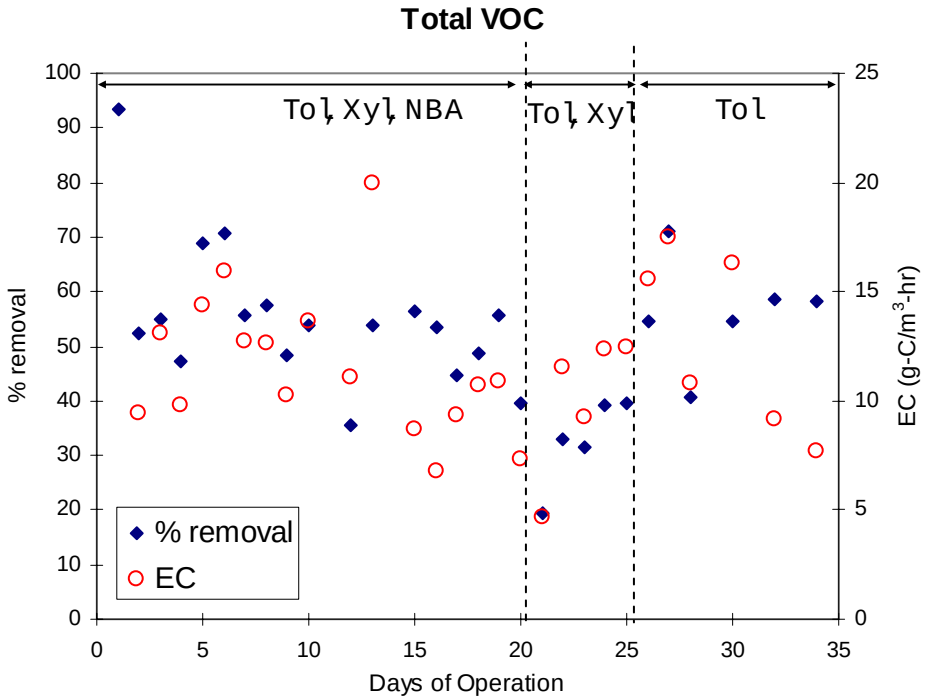


Figure 8-6: p-xylene removal efficiency and elimination capacity during the start-up period.

The initial VOC removal observed in the pilot-scale bioreactor was relatively low compared to the results obtained with the laboratory-scale system. Uneven distribution of the inlet gas stream or the nutrient solution through the pilot bioreactor could have contributed to the poorer removal observed in the pilot-scale biofilter. Such problems are common when scaling a system up from laboratory to the pilot scale. Another possible reason for poor removal was that insufficient biomass had established itself on the biofilter packing media during this startup period. On day 28, only approximately 35 mg COD/g-foam was present on the foam packing in the pilot bioreactor. In the previous laboratory-scale experiments discussed in Chapter 4, overall VOC removal was only 70% when the biomass quantity on the foam was approximately 50 mg COD/g-

foam. Thus, the overall VOC removals initially observed in the pilot reactor are consistent with those expected when relatively low biomass quantities are present in the bioreactor. However, the previous laboratory-scale results also suggested that higher biomass quantities, nutrient hold-up, and ultimately VOC removals would be expected when the ketone components of the waste gas stream were introduced to the bioreactor. Thus, even though removal efficiency of aromatic compounds was relatively low (60% of the inlet toluene and 30% of the inlet p-xylene) at the end of the startup period, the actual paint spraying experiment was initiated in the bioreactor because the aromatic compounds constitute only a small fraction of the paint VOC mixtures (less than ~10% by mass).

8.2.2 Phase I: Baseline Paint Spraying Experiments (Day 1- Day 8)

After the biofilter had been continuously supplied with a surrogate waste gas stream containing n-butyl acetate, p-xylene, and toluene for approximately one month, the biofilter was fed the off-gas stream from actual paint spraying operations for 6 hours per day. Because ketone compounds made up the highest fraction of the waste gas stream and the biofilter had not been pre-acclimated to this compound during the startup phase, the ketone removal efficiency in the bioreactor was low for the first few days of painting operation. To improve bioreactor performance, fresh nutrients were provided to the column and the system was provided with a supplemental slip feed. The merits of using a supplemental (slip feed) system to improve bioreactor performance had been demonstrated earlier in a previous study (Park and Kinney 2001).

In such a slip feed system, a surrogate carbon source is added to the biofilter during the off period when air but no waste gas VOCs are passing through the biofilter. In previous slip feed experiments with laboratory-scale bioreactors, a small quantity of a

surrogate carbon source was supplied continuously to the biofilter in the gas phase during the shutdown period (Park and Kinney 2001). However, this feed method required additional feed lines that add to the complexity of the biofilter system. For this reason, the design of the slip feed system was modified during the pilot-scale tests. That is, a single spike of a surrogate carbon source was added to the liquid nutrient media that was recirculated through the biofilter during the booth off period. In this case, a total of 6 g of methyl isoamyl ketone, MIAK (as carbon) was injected once as a neat liquid into the bioreactor sump during the 18-hour period when no paint emissions were provided to the bioreactor. MIAK was selected for the slip feed because it constituted the greatest fraction of the paint off-gas stream.

Figure 8-4 summarizes the VOC removal efficiency observed in the bioreactor over the 6-hour operational period the next day. The biofilter recovered ketone degradation activity and achieved approximately 90% removal of the total VOC mixture by the end of the 6- hour painting period (Figure 8-7 and Table 8-4).

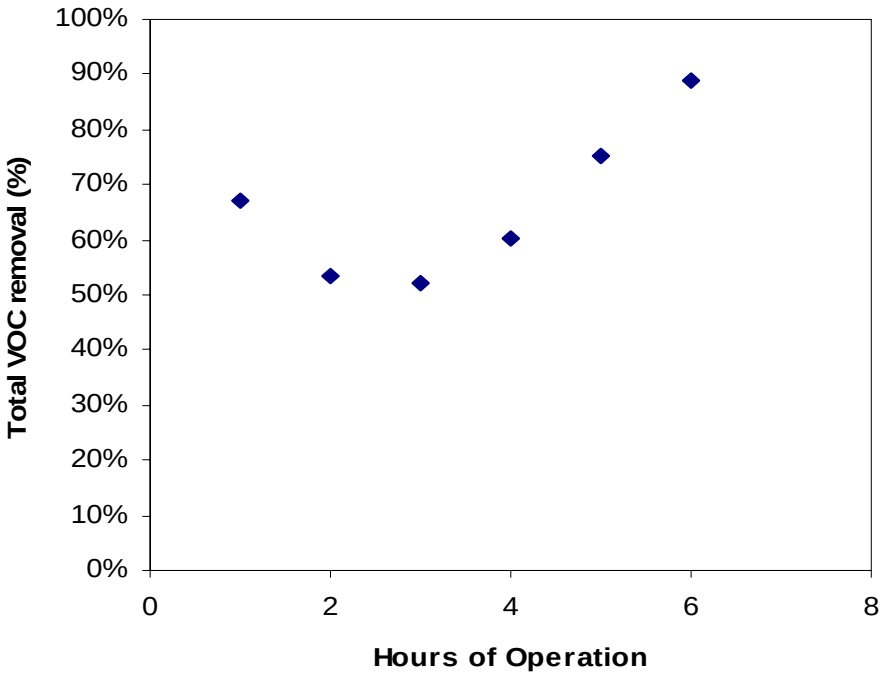


Figure 8-7: Removal of paint VOCs in the bioreactor during the third day of painting operations. (Note: Fresh nutrients and an MIAK spike were added to the bioreactor during the 18 hour off period preceding the beginning of painting operations, denoted as time zero)

Table 8-4: Speciated VOC removal on the sixth hour of painting operations on Day 3.

Compound	Concentration (ppm_v)		% VOC removal
	Inlet	Outlet	
MEK	15.51	0.00	100.00%
MIBK	5.70	0.77	86.44%
Toluene	4.64	1.52	67.16%
NBA	3.44	0.00	100.00%
MIAK	24.65	2.79	88.69%
p-xylene	2.25	1.24	44.72%
MAK	1.01	0.00	100.00%
Total VOCs	57.21	6.33	88.94%

Table 8-5 summarizes the VOC removals observed on Day 4 (two days after the MIAK spike had been added to the bioreactor). Unlike the Day 3 results shown in

Figure 8-4, the VOC removal efficiency during the first and second hour of paint spraying remained stable (data not shown) indicating that the biodegradation capabilities of the bioreactor had improved.

Table 8-5: Average VOC removal efficiency during the 6-hour painting period on Day 4 of painting operations.

Compound	Concentration (ppm_v)		% VOC removal
	Inlet	Outlet	
MEK	12.69	0.00	100.00%
MIBK	2.51	0.00	100.00%
Toluene	0.68	0.14	79.09%
NBA	2.27	0.00	100.00%
MIAK	11.07	0.00	100.00%
p-xylene	1.45	1.04	27.85%
MAK	0.02	0.00	100.00%
Total VOCs	30.69	1.19	96.14%

During the following weekend (Days 5 and 6), no paint was sprayed, however, a surrogate VOC stream including 8 g-C/m^3-hr n-butyl acetate and 4 g-C/m^3-hr of toluene was supplied to the column to see if this combination of VOCs could maintain biomass activity during the shutdown period. A single spike of 12 g-C of MIAK liquid was also added to the column sump during the weekend shutdown. Table 8-6 summarizes the VOC removal observed in the biofilter two hours after resuming a 200 ppm_v paint spray feed to the biofilter following the weekend shutdown period. Since the biofilter had been supplied with toluene throughout the weekend, higher removals of the aromatic compounds toluene and xylene were observed. However, relatively low removal of the ketone constituents of the waste gas stream was also observed suggesting that the single MIAK spike was insufficient to maintain biomass activity over a weekend shutdown period. The column seemed to require a longer time to recover ketone degradation

activity following the weekend shutdown than it did following its daily 18-hour shutdown; however, due to a mechanical clogging problem with the paint spray gun, the system could not be observed for longer duration on that day. Thus, the length of time necessary to fully recover ketone degradation was not determined.

Table 8-6: VOC removal two-hours after resuming painting operations following a weekend shutdown.

Compound	Concentration (ppm_v)		% VOC removal
	Inlet	Outlet	
MEK	47.42	14.57	69.28%
MIBK	18.47	5.12	72.25%
Toluene	6.62	0.48	92.71%
NBA	16.66	0.00	100.00%
MIAK	100.61	38.46	61.77%
p-xylene	16.75	2.45	85.36%
MAK	1.19	0.00	100.00%
Total VOCs	207.72	61.09	70.59%

8.2.3 Phase II: Paint spraying experiments (No MIAK slip feed; Days 11 –15)

Following the weekend shutdown period (Days 9 and 10) when small amounts of toluene and MIAK were provided to the biofilter as a slip stream, the biofilter was operated for six hours per day treating paint spray emissions followed by an 18 hr off period when only air was provided to the biofilter. The response of the biofilter during the paint spray feed period is summarized in Figure 8-8 for Days 11 through 15 of operation. During this period of operation, no MIAK was supplied during the daily off period. As evident in the Figure 8-8, the biofilter initially had difficulty removing the paint mixture. This likely resulted because it had been provided only a small quantity of MIAK during the weekend shutdown period. In fact, the MIAK spike added to the biofilter sump during the weekend shutdown period was completely consumed within

two hours. Nevertheless, the ketone degradation capacity of the bioreactor recovered by the second day following the weekend shutdown, and an overall VOC removal efficiency of 85 % was achieved. However, this removal was not maintained over the next few days of operation (see Figure 8-8) most likely due to the short operating period (i.e., 6 hours per day) that the bioreactor was actually provided a carbon source. These results suggest that it will likely be necessary to employ a slip feed system to maintain VOC degradation activity in the biofilter during the booth off periods, at least during startup, in applications where higher removal efficiencies are required.

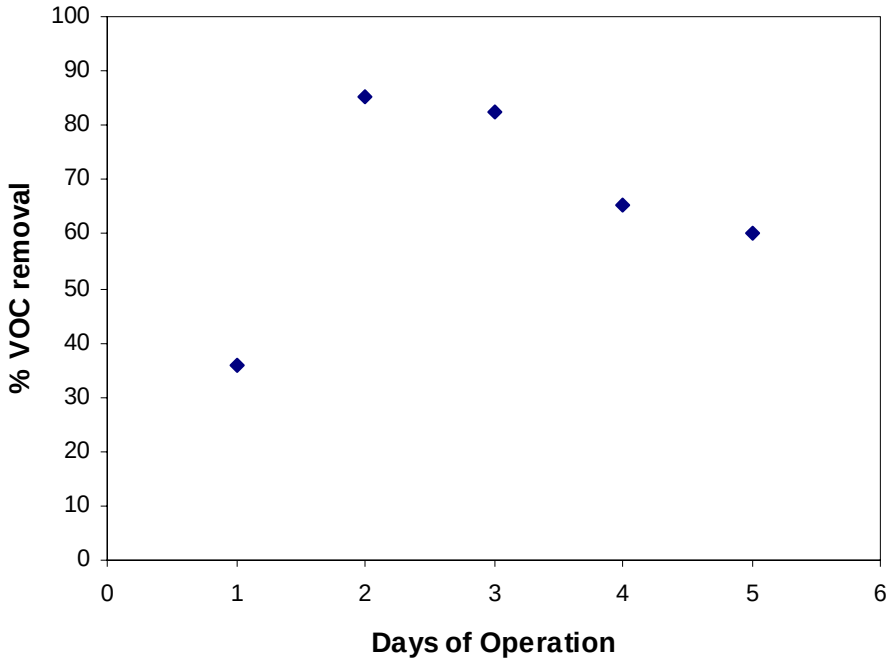


Figure 8-8: Overall VOC removal efficiencies in the biofilter when no MIAK slip feed was provided during the booth off-period.

8.2.4 Phase III: Paint spraying experiments (MIAK slip feed provided; Days 28 to Day 38)

Due to technical problems in the paint spray booth, the operation of the paint spray booth was discontinued for approximately 10 days following the Phase II experiments described above. During this period, a surrogate paint mixture consisting of toluene, methyl isoamyl ketone (MIAK), and p-xylene was supplied to the biofilter. Following this surrogate feeding period, the biofilter was again exposed to the off-gas stream from the paint spray booth for Phase III experiments. The paint spray booth was operated for 6 hours per day, 5 days per week in a manner that mimicked actual paint spray booth operation. Figure 8-9 shows the overall VOC removal efficiency obtained throughout this phase of the experiments.

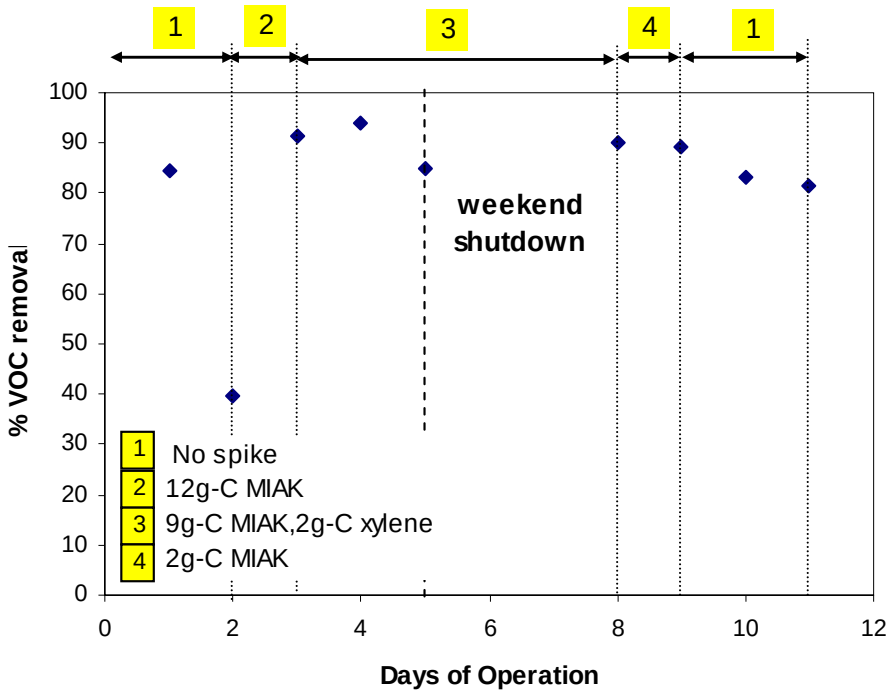


Figure 8-9: VOC removal during the Phase 3 paint spraying experiments.
(Note: the MIAK provided during each 18hr booth off period is indicated in the legend)

As shown in Figure 8-9, the initial response of the biofilter with respect to removal of actual paint VOCs was quite rapid (i.e., ~85% VOC removal). Biofilter performance was not adversely affected by the 18-hours of starvation prior to the painting experiments since the biofilter had been continuously supplied with a surrogate paint VOC mixture for approximately 10 days prior to the 18-hr starvation period. However, during the next 18-hour off period, the biofilter lost its VOC degradation capacity and the average VOC removal during the next 6 hours of painting (Day 2 in Figure 8-9) was only 40%. The short period of carbon supply (i.e., 6 hours with a carbon source and 18 hours without a carbon source) was insufficient to maintain the VOC degradation activity of microorganisms in the biofilter. To overcome this activity loss in the microbial population, a slip feed system was introduced during the off period of biofilter operation. As can be seen in Figure 8-9, injection of 12 g-C MIAK liquid to the recirculating nutrient solution during the off period increased the VOC removal efficiency to over 90% when the biofilter was restarted the next day.

The mass of the MIAK spike provided during the off period was varied to determine whether it had a strong effect on the effectiveness of the slip feed system. Results demonstrated that VOC spikes as low as 2 g were sufficient to maintain microbial biodegradation activity. Biofilter response was rapid when the system was re-exposed to the VOC-laden waste gas stream following the 18 hour paint booth off period. Interestingly, a daily MIAK spike during the weekend shutdown period was sufficient to maintain VOC degradation activity in the biofilter following the weekend shutdown, while a one time injection of MIAK spike over the weekend shutdown period during the paint spray experiments in Phase II was not sufficient to maintain VOC degradation activity.

The quantity of biomass present in the foam packing material during Phase III experiments (e.g., 140 mg COD/ g-foam) was nearly double that present during the Phase II experiments. To determine whether increased biomass alone or the MIAK slip feed system was responsible for the improvement in the biofilter performance observed during the Phase III experiments, no MIAK spike was provided during the last two days of the experiments. The overall VOC removal efficiency decreased over time after the MIAK spike was discontinued indicating that the MIAK slip feed system was important to maintain the biodegradation capacity in the biofilter. This result conflicts somewhat with the results obtained in the laboratory-scale experiments where a slip feed system was not required to maintain high VOC removal efficiencies during booth shutdown periods (see Chapter 7). One possible reason for this discrepancy is that the biomass quantity in the pilot-scale biofilter at the time of the Phase III experiments was only half of that present in the laboratory-scale system which had been operating for almost 200 days. The laboratory-scale biofilter experiment also indicated that biomass quantity is strongly related to nutrient retention capacity and overall VOC removal achievable in the system. These results suggest that as additional biomass is established in the pilot-scale biofilter with time, a more stable VOC removal could be expected and an external slip feed system may not be necessary.

8.2.5 Biofilter Response as a Function of VOC Concentration

Since the quantity of VOCs emitted from the paint spray booth varied greatly depending on even slight changes to the paint spray gun controller, the VOC loading to biofilter varied widely during the pilot biofilter experiments. Thus, these emissions mimic the widely varying emissions from actual paint spray booths. Nevertheless, it was of interest to determine how the pilot biofilter responded to high and low

concentrations of paint VOCs. Table 8-10 presents the average VOC removal observed in the biofilter when the paint VOC emissions were low (i.e., less than 15 ppm_v total). As evident from the table, nearly all of the VOCs generated from the paint spray booth operation were degraded in the biofilter except for the aromatics toluene and xylene.

Table 8-7: Average VOC removal efficiency during the 6-hour painting period on Day 31 of operation.

Day 31	Concentration (ppm_v)		% VOC removal
Compound	Inlet	Outlet	
MEK	4.75	0.00	100.0%
MIBK	1.45	0.00	100.0%
Toluene	0.50	0.17	66.7%
NBA	1.39	0.00	100.0%
MIAK	4.82	0.00	100.0%
p-xylene	1.01	0.67	33.2%
MAK	0.02	0.00	100.0%
Total VOC	13.94	0.84	94.0%

High VOC removals (i.e., greater than 90%) were achieved when the biofilter treated paint spray emissions with low inlet concentrations (i.e., less than 15 ppm_v). Approximately 80% of the total VOCs applied to the biofilter were degraded in the first section of the biofilter (see Figure 8-10).

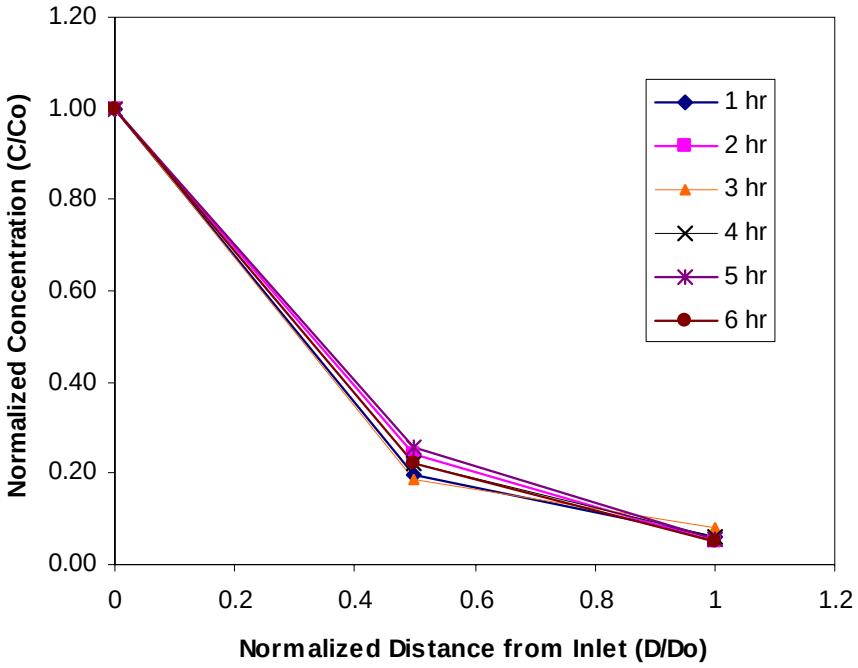


Figure 8-10: VOC removal profile across the bioreactor treating a 14 ppmv inlet concentration on Day 31 of operation.

The biofilter could also achieve reasonable removal of paint VOCs while treating high concentrations of VOCs (Table 8-8 and Figure 8-11).

Table 8-8: Average VOC removal efficiency during the 6-hour painting period on Day 32 of operation.

Compounds	Concentration (ppm_v)		% VOC removal
	Inlet	Outlet	
MEK	43.53	0.45	99.0%
MIBK	13.10	1.60	87.8%
Toluene	3.78	2.25	40.6%
NBA	8.88	0.00	100.0%
MIAK	51.47	8.83	82.8%
p-xylene	7.19	6.18	14.0%
MAK	0.48	0.00	100.0%
Total VOC	128.42	19.31	85.0%

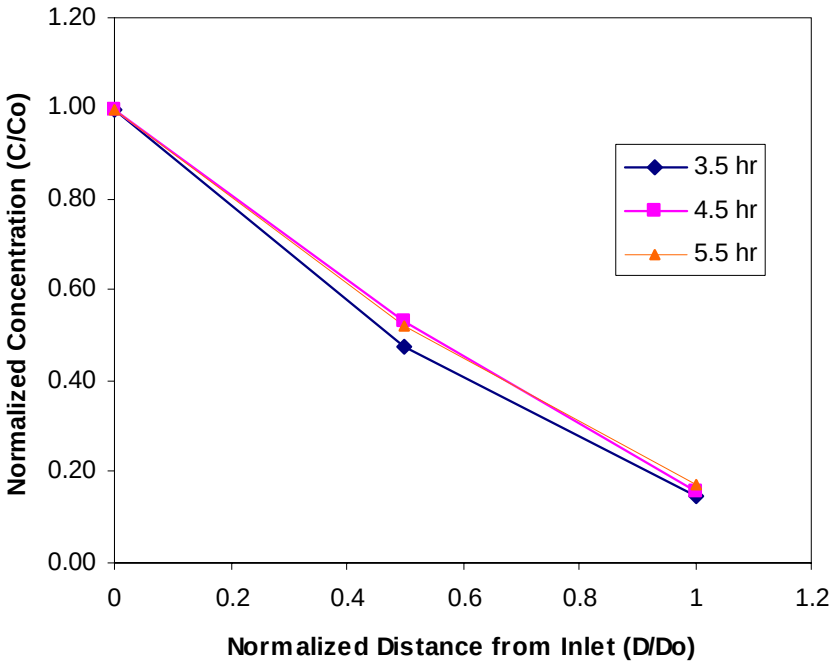


Figure 8-11: VOC removal profile across the bioreactor treating VOCs with a total inlet concentration of 128 ppmv on Day 32 of operation.

These results indicate that within the range of VOC concentrations tested, the biofilter could successfully degrade paint VOC mixtures under realistic feed conditions. Generally, the biofilter could easily degrade mixtures of ketone compounds and acetates without any evidence of strong substrate inhibitions. Just as was observed in the lab-scale tests, however, biodegradation of the aromatic hydrocarbons was much lower than the other VOCs. The ratio of the concentration of the aromatic hydrocarbons to the total of all VOCs in the paint emissions, however, was relatively low and thus the biofilter still achieved high VOC removals overall in spite of the low removal for aromatics. Depending on the treatment goal, the bioreactor can be configured as two separate bioreactor columns in series to improve the aromatic degradation in the biofilter. The

first bioreactor would be inoculated and maintained for the degradation of the ketone constituents of the waste gas stream and the second bioreactor could be inoculated and maintained to sustain the aromatic hydrocarbon degrading population. This segregation of the microbial culture may enhance the aromatic degradation since the slip feed to the bioreactor could be optimized for each bioreactor individually.

8.3 SUMMARY

The feasibility of biofiltration for paint spray booth applications was investigated in the pilot-scale bioreactor under realistic feeding conditions. The results indicate that the biofilter could successfully treat the off-gas stream from a paint spray booth containing a mixture of ketones. Degradation of the aromatics toluene and xylene limited the overall VOC removal obtainable by the biofilter. The results also indicate that until the biofilm was strongly established on the packing media in the biofilter, a surrogate carbon supply (i.e., slip stream) was necessary during the off period to maintain the VOC degradation activity of the microorganism in the biofilter under transient feeding conditions. In addition, within the VOC concentration ranges tested (10 ppm_v to 200 ppm_v); the biofilter can achieve reasonable VOC removals of greater than 85% under realistic feeding conditions in which the inlet VOC concentrations vary daily and hourly.

Chapter 9 Conclusions

The main objectives of this research were to investigate the feasibility of biofiltration for paint spray booth applications and to delineate how key operating parameters and biofilter history affect the degradation of paint VOC mixtures and the microbial population in biofilters. The conclusions that can be drawn from this study are as follows:

- Biofilters are a feasible option for treating the emissions from paint spray booth facilities. Removal efficiencies as high as 98% were achieved in laboratory scale biofilters under steady and intermittent feed conditions typical of booth operation. For VOC concentrations on the order of 10 to 100 ppmv, high removals could be achieved at gas phase residence times as low as 15 sec. In a pilot scale biofilter treating actual paint emissions on an intermittent schedule, removals as high as 96% were achieved. Generally, the removal of the aromatic components of the paint mixture limits the overall VOC removals achievable in biofilters treating paint mixtures. Enriching the microbial population capable of degrading aromatic hydrocarbons via a sequential feed strategy was found to be beneficial for achieving high removals of these compounds.

- When the nitrogen supply was limited, poor removal was observed during the start-up period in a biofilter treating a paint VOC mixture. Degradation of the aromatic compounds in particular was severely repressed. Even though nutrients are a crucial factor for facilitating microbial growth and improving pollutant removal in biofilter columns, it is difficult to achieve nutrient (nitrogen) rich

conditions in polyurethane foam biofilters during the start-up period. However, a sequential feeding strategy can help establish the biomass in the column, which in turn, can increase the nutrient hold-up in a polyurethane foam biofilter.

- Although a sequential feed strategy can improve degradation of the aromatics in a mixture, enriching the culture used to initially inoculate the bioreactor is sufficient to improve biofilter performance. To preserve as much of the diversity of the VOC-degrading microbial population as possible, the mixed microbial culture used to inoculate the biofilters was individually enriched for microorganisms capable of degrading each VOC component of the mixture. In this case, even the continuously fed column (CFC) achieved high paint VOC removal efficiencies without significant substrate inhibition but the supply of nutrient was still found to be crucial for achieving high removals. Generally, when the inoculating culture was developed so as to maintain the degradation capacity of the culture for each VOC, the sequential feeding strategy did not appear to provide any initial advantage with respect to VOC removal; however, the system ultimately achieved higher aromatic hydrocarbon degrading capacity.

- The DGGE banding pattern results indicate that the mixed culture used to inoculate the biofilters was predominantly bacterial but several of the bacterial species initially present in the inoculum disappeared shortly after being transferred to the bioreactor. These results confirm that some bacterial species from a well-mixed batch system may not survive in the biofilm of a bioreactor where the environmental conditions and microbial competition are likely more challenging.

- The sequential and continuous feeding strategies led to the development of similar bacterial and fungal populations in the biofilters, if one considers the community that developed across each bioreactor as a whole. However, the microbial population that developed in a given section of each biofilter (e.g., top section) was generally different from the population that developed in the corresponding section of the other biofilter. Generally, the bacterial population present was spatially diverse across each biofilter and the DGGE results suggest that this population became less diverse with operating time. In contrast, the fungal species in the column were relatively uniform across the column and also quite stable over time. Differences in the composition of the bacterial population were found to more closely reflect changes in the VOC removal profile suggesting that the bacteria were the primary VOC degraders in this system.

- The DGGE analyses indicate that the bacterial population was greatly affected by the change in the nitrogen conditions within the column, while the fungal population was much more stable with time and spatially more uniform than the bacterial population in the biofilter regardless of nitrogen availability. Fewer bacterial DGGE bands were observed under nitrogen rich conditions than under nitrogen limited condition. Under nitrogen rich conditions, a few faster growing organisms may dominate the culture and be selectively amplified in the DNA extraction and PCR steps. As a result, only a few bands representative of those organisms that grow fastest under nitrogen rich conditions would appear on the gels. However, under nitrogen limited conditions, the N-rich competitive organisms would not be able to compete with the N-limited competitive

organisms. Since N-rich organisms would no longer dominate the culture, it is possible to extract and amplify DNA from the variety of species present. Nonetheless, PCR amplification of DNA and DGGE analysis of the products has provided a useful means to directly characterize the bacterial (and fungal) populations present within operating bioreactors.

- The SFC and CFC biofilters which had been continuously operated for more than 200 days were quite resilient to transient loading conditions and tolerated shutdown periods up to 9 days. No additional supply of carbon was required during the off period to maintain the pollutant degradation capacity. In fact, the performance of the SFC and CFC bioreactors improved as they became acclimated to operating under transient load conditions. These results suggest that well-established biofilters with mature biofilms are resilient to intermittent feed conditions.

- The feasibility of biofiltration for paint spray booth applications was investigated in the pilot-scale bioreactor under realistic feeding conditions. The results indicate that biofilters can successfully treat the off-gas streams from paint spray booth containing a mixture of ketones within the VOC concentration ranges tested (10 ppm_v to 200 ppm_v), a biofilter can achieve reasonable VOC removals of greater than 85% under realistic feeding conditions in which the inlet VOC concentrations vary daily and hourly. Degradation of the aromatics toluene and xylene limited the overall VOC removal obtainable by the biofilter. The results also indicate that until the biofilm was well established on the packing media in the biofilter, a surrogate carbon supply (i.e., slip stream) was necessary during the

off period to maintain the VOC degradation activity of the microorganisms in the biofilter under transient feeding conditions. Further studies will be required to optimize the surrogate feed system for field-scale systems.

While this research focused on paint VOC mixtures, it is anticipated that many of the phenomena observed will be common to other VOC contaminated waste gases. The work presented in this dissertation has delineated the effect that nutrient availability and VOC acclimation history have on the microbial community and the degradation capacity of bioreactors treating VOC mixtures. These factors which control the biofilter performance are so closely related that they must be considered together for the successful application of biofilter technology for VOC mixtures. Even though pure culture studies might be helpful to understand fundamental questions in a biological system, they do not represent the properties of the complex microbial communities found in actual treatment systems. Moreover, the functioning of each microbial species in a mixed culture may vary as a function of the environmental conditions present in a bioreactor system. In this study, a PCR-DGGE technique was used to monitor how the diversity of the microbial community fluctuated in a biofilter system over an extended period of operation. The relationship between operating parameters such as nitrogen availability, feeding strategy and microbial community were successfully investigated using this technique. However, the DGGE technique is not well suited for quantification even though this technique can reveal the presence or absence of bacterial and fungal species in a biofilm sample. Thus, it would be useful in future studies to couple the DGGE technique with a quantitative measurement of the specific microbial species of interest to better understand their function in a complex microbial community.

Regarding to the feasibility of biofilter application in the field, this study indicates that surrogate feed addition during shutdown periods may be necessary for maintaining the pollutant degradation capacity of biofilters under field conditions when shutdown and restart events are frequent. In particular, the minimal amount of surrogate feed required and the optimal injection method should be delineated for successful application of biofilters in the field.

Bibliography

Acinas G, Rodriguez-Valera F, Pedros-Alio C (1997) “Spatial and temporal variation in marine bacterioplankton diversity as shown by RFLP fingerprinting of PCR amplified 16S rDNA.” *FEMS Microbial Ecology* **24**: 27-40.

Acuna, M. E., Villanueva, C., Cardenas, B., Christen, P., and Revah, S. (2002). "The effect of nutrient concentration on biofilm formation on peat and gas phase toluene biodegradation under biofiltration conditions." *Process Biochemistry* **38**: 7-13.

Aizpuru, A. e. a. (2001). "Biofiltration of a Mixture of Volatile Organic Emissions." *Journal of Air & Waste Management Association* **51**: 1662-1670.

Alvarez, P. J. J., and Vogel, T. M. (1991). "Substrate Interactions of Benzene, Toluene, and para-Xylene during Microbial Degradation by Pure Cultures and Mixed Culture Aquifer Slurries." *Applied and Environmental Microbiology* **57**(10): 2981-2985.

Amann R I., L. W., and Schleifer K H. (1995). "Phylogenetic identification and in situ detection of individual microbial cells without cultivation." *MIcrobiological Reviews* **59**(1): 143-169.

American Public Health Association, A. W. W. A., Water Environment Federation (1992). Standard Methods for the Examination of Water and Wastewater. Washington, D.C.

Anniston Army Depot (2000). VOC and HAP Emissions Inventory for Bldg 409, Booths A, B and C, 0/01/00-12/31/00.

Apel, W.A., Barnes, J.M., and Barrett, K.B. (1995), “Biofiltration of nitrogen oxides from fuel combustion gas stream,” *Proc. 88th Ann. Meeting of the Air & Waste Manage. Assn.*, San Antonio, Texas.

Barshter, D.W., Paff, S.W., and King, A.B. (1993), “Biofiltration: Room Temperature Incineration,” *Proc. 86th Ann. Meeting of the Air & Waste Manage. Assn.*, Denver, Colorado.

Barton, J. W., Klasson, T., and Davison, B.H. (1997). “Extended Operation and Control of Biomass Overgrowth in Biofilters Designed for VOC removal.” *Air & Waste Management Association*, Toronto, Ontario, Canada.

Bhattacharya, S. a. B., B.C. (2001). “Long Term Operation and Performance of a Biotrickling filter Treating ortho-Dichlorobenzene and Ethanol Mixtures.” *Air & Waste Management Association*, Orlando, Florida.

Boswell, J. e. a. (2001). “Biofiltration of VOCs from Paint Manufacturing.” *Air & Waste Management Association*, Orlando, Florida.

Bruggemann J., Stephen J.R., Chang Y.J., Macnaughton S.J., Kowalchuck, G.A., Kline, E. and White D.C. (2000) “Competitive PCR-DGGE analysis of bacterial mixtures as an internal standard and an appraisal of template enumeration accuracy.” *Journal of Microbiological Methods* **40**:111-123.

Burback, B. L., and Perry, J.J. (1993). "Biodegradation and Biotransformation of Groundwater Pollutant Mixtures by *Mycobacterium vaccae*." *Applied and Environmental Microbiology* **59**(4): 1025-1029.

Casamayor, E.O., Schafer, H., Baneras, L., Pedros-Alio, C., and Muyzer G. (2000). “Identification of and spatio-temporal differences between microbial assemblages from tow neighboring sulfurous lakes: comparison by microscopy and denaturing gradient gel electrophoresis.” *Applied and Environmental Microbiology*. **66**:499-508.

Castro, A. D., Allen, D.G.G., and Fulthorpe, R.R. (1997). “Characterization of the Microbial Population During Biofiltration and the Influence of the Inoculum Source.” *Air & Waste Management Association*, Toronto, Ontario, Canada.

Chang, M. K., Voice, T.C., and Criddle, C.S. (1992). "Kinetics of Competitive Inhibition and Cometabolism in the Biodegradation of Benzene, Toluene, and p-Xylene by Two Pseudomonas Isolates." *Biotechnology and Bioengineering* **41**: 1057-1065.

Chemfinder (2003). www.chemfinder.com.

Choi, D., Devinney, J.S., and Deshusses, M.A. (1998). “Performance of a Pilot Scale Biotrickling Filter Under Non-Steady State Conditions.” USC-TRG Conference on Biofiltration, Los Angeles, California.

Corsi, R. L., and Seed, L. (1995). "Biofiltration of BTEX: Media, Substrate, and Loadings Effect." *Environmental Progress* **14**(3): 151-158.

Cox, H.H.J. and Deshusses, M.A. (1997), “The Use of Protozoa to Control Biomass Growth in a Biological Trickling Filter for Waste Air Treatment,” *Proc. 90th Ann. Meeting of the Air & Waste Manage. Assn.*, Toronto, Canada.

Deeb, R. A., Alvarez-Cohen, L. (1999). "Temperature Effects and Substrate Interactions During the Aerobic Biotransformation of BTEX Mixtures by Toluene-Enriched Consortia and *Rhodococcus rhodochrous*." *Biotechnology and Bioengineering* **62**(5): 526-536.

Deeb, R. A., Hu, H.-Y., Hanson, J.R., Scow, K.M., and Alvarez-Cohen, L. (2001). "Substrate Interactions in BTEX and MTBE Mixtures by an MTBE-Degrading Isolate." *Environmental Science & Technology* **35**: 312-317.

Delhomenie, M. C. e. a. (2001). "Influence of nitrogen on the degradation of toluene in a compost-based biofilter." *Journal of Chemical Technology and Biotechnology* **76**: 997-1006.

Demeestere K., H. Van Langenhove, E. Smet. (2002). "Regeneration of a compost biofilter degrading high loads of ammonia by addition of gaseous methanol." *Journal of the Air and Waste Management Association.* **52**(7), pp.796-804.

Deshusses, M. a. Johnson, C.T (1999). "Biofiltration of High Loads of Ethyl Acetate in the Presence of Toluene." *Journal of Air & Waste Management Association* **49**: 973-979.

Deshusses, M. A. and Johnson, C. T. (2000) "Development and Validation of a Simple Protocol to Rapidly Determine the Performance of Biofilters for VOC Treatment," *Environmental Science & Technology* 34, 461-467.

Devinny, J.S., Deshusses, M.A., and Webster, T.S. (1999), *Biofiltration for Air Pollution Control*. CRC Press.

du Plessis, C. A., Strauss, J.M., and Riedel, K.-H.J. (2001). "BTEX catabolism interactions in a toluene-acclimatized biofilter." *Applied Microbiology and Biotechnology* **55**: 122-128.

Ergas, S.J., Schroeder, E.D., Change, D.P.Y., and Morton, R.L.(1995), "Control of Volatile Organic Compound Emissions Using a Compost Biofilter," *Water Environment Research*, 67(5), pp.816-821.

Fantroussi, S. E., *et al* (1999). "Bioaugmentation of a Soil Bioreactor Designed for Pilot-Scale Anaerobic Bioremediation Studies." *Environmental Science & Technology* **33**: 2992-3001.

Fernandez, A. e. a. (1999). "How Stable Is Stable? Function versus Community Composition." *Applied and Environmental Microbiology* **65**(8): 3697-3704.

Fernandez, A. e. a. (2000). "Flexible community Structure Correlates with Stable Community Function in Methanogenic Bioreactor Communities Perturbed by Glucose." *Applied and Environmental Microbiology* **66**(9): 4058-4067.

Friedrick, U., H. Van Langenhove, K. Altendorf and A. Lipski. (2003). “Microbial Community and Physicochemical Analysis of an Industrial Waste Gas Biofilter and Design of 16S rRNA targeting oligonucleotide probes.” *Environmental Microbiology*. 5(3): 183-201.

Ft. Hood, Personal Communication with Painting Personnel, February, 2004.

Gribbins, M. J., and Loehr, R.C. (1998). "Effect of Media Nitrogen Concentration on Biofilter Performance." *Journal of Air & Waste Management Association* **48**: 216-226.

Haack, S. K., Garchow, H., Odelson, D. A., Forney, L. J., and Klug, M. J. (1994). "Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities." *Applied and Environmental Microbiology* **60**(7): 2483-2493.

Hamer, G. a. T., A. (2001). “The Biotreatment of Acetone and N-Propanol Vapors.” *Air & Waste Management Association*, Orlando, Florida.

Haruta, S., Cui, Z., Haung, Z.,Li, M., Ishii, M., Igarashi, Y. (2002a). "Construction of a stable microbial community with high cellulose-degradation ability." *Applied Microbiology and Biotechnology* **59**: 529-534.

Haruta, S., Kondo, M., Nakamura, K., Aiba, H., Ueno, S., Ishii, M., and Igarashi, Y. (2002b). "Microbial community changes during organic solid waste treatment analyzed by double gradient denaturing gradient gel electrophoresis and fluorescence in situ hybridization." *Applied Microbiology and Biotechnology* **DOI 10.1007/s00253-002-1074-9**.

Head, I. M., Saunders, J. R., and Pickup, R. W. (1998). "Microbial evolution, diversity and ecology: a decade of ribosomal RNA analysis of uncultivated microorganisms." *Microbial Ecology* **35**(1): 1-21.

Hodge and F.E. Reynolds, Jr.Hodge, D.S., Medina, V.F., Islander, R., and Devinny, J.S. (1991), “ Biofiltration of Hydrocarbon Fuel Vapors in Biofiltration,” *Environmental Technology*, 12, pp.665-662.

Holubar, P., Andorfer, C., and Braun, R. (1999). "Effects of nitrogen limitation on biofilm formation in a hydrocarbon-degrading trickle-bed filter." *Applied Microbiology and Biotechnology* **51**: 536-540.

Hsu, S. K., Shen, K.P., Lin, S.S., Wang, Y.M., and Chen, H.W. (2000). “Biofilter Application for Control of Volatile Organic Compounds (VOC) from Paint Manufacturing Industry.” *Air & Waste Management Association*, Salt Lake City, Utah.

Jorio, H., Bibeau, L., and Heitz, M. (2000). "Biofiltration of Air Contaminated by Styrene: Effect of Nitrogen Supply, Gas Flow Rate, and Inlet Concentration." *Environmental Science & Technology* **34**(9): 1764-1771.

Jorio, H., Kiared, K., Brzezinski, R., Leroux, A., Viel, G., and Heitz, M. (1998). "Treatment of Air Polluted with High Concentrations of Toluene and Xylene in a Pilot-Scale Biofilter." *Journal of Chemical Technology and Biotechnology*. **73:** 183-196.

Kar, S., Swaminathan, T., and Baradarajan, A. (1996). "Studies on biodegradation of a mixture of toxic and nontoxic pollutant using Arthrobacter species." *Bioprocess Engineering* **15**: 195-199.

Kazenski, S. L., and Kinney, K.A. (2000). "Biofiltration of Paint Spray Booth Emissions:Packing Media Considerations and VOC Interactions." *Air & Waste Management Association,* Salt Lake City, Utah.

Kim, B. R. e. a. (2000). "Biological Removal of Gaseous VOCs from Automotive Painting Operations." *Journal of Environmental Engineering* **126**(8): 745-753.

Kinney, K. A., Loehr, R.C., and Corsi, R.L. (1999). "Vapor-Phase Bioreactors: Avoiding Problems Through Better Designs and Operation." *Environmental Progress* **18**(3): 222-230.

Kok, H.J.G. (1992), "*Bioscrubbing of Air Contaminated with High Concentrations of Hydrocarbons,"* in Dragt A.J. and Ham J. Van (Eds.) Biotechniques for Air Pollution Abatement and Odour Control Policies, *Proceeding of an International Symposium*, Maastricht, The Netherlands, October 27-29, 1991, pp. 77-82. Elsevier, Amsterdam.

Lee, E. Y., Jun, Y.S., Cho, K,S., and Ryu, H.W. (2002). "Degradation Characteristics of Toluene, Benzene, Ethylbenzene, and Xylene by *Stenotrophomonas maltophilia* T3-c." *Journal of Air & Waste Management Association* **52**: 400-406.

Leson, G. a. W., A.M. (1991). "Biofiltration: An Innovative Air Pollution Control Technology for VOC Emissions." *Journal of Air & Waste Management Association* **41**(8): 1045-1054.

Leson, G., and Winer, A.M. (1991). "Biofiltration: An Innovative Air Pollution Control Technology for VOC Emissions." *Journal of Air & Waste Management Association*, **41**(8), 1045-1054.

Li, C.;and Moe, W.M. (2003) "Sequencing Batch Biofilter Operation for Treatment of Methyl Ethyl Ketone (MEK) Contaminated Air," *Environmental Technology*, **24**(5), 531-544.

Lovanh, N., Hunt, C.S., Alvarez, P.J.J. (2002). "Effect of ethanol on BTEX biodegradation kinetics: aerobic continuous culture experiments." *Water Research* **36**: 3739-3746.

Lu,C., *et al*., (2001), " Removal of Pentane and Styrene Mixtures from Waste Gases by a Trckle-Bed Air Biofilter," *J.Chem. Technol, Biotechnol*., **76**(8), pp 820-826, Society of Chemical Industry, August 2001.

Luxmy, B. S., Nakajima, F., and Yamamoto, K. (2000). "Analysis of bacterial community in membrane-separation bioreactors by fluorescent in situ hybridization (FISH) and denaturing gradient gel electrophoresis techniques." *Water Science and Technology* **41**(10-11): 259-268.

Madigan, M. T., Martinko, J.M., and Parker., J. (2000). Brock Biology of Microorganisms, Prentice Hall.

Martin, F. J. a. L., R.C. (1996). "Effects of Periods of Non-Use on Biofilter Performance." *Journal of Air & Waste Management Association* **46**: 539-546.

McMinn, B. W., Newman, C.R., McCrillis, R.C., and Kosusko, M. (1992). VOC Prevention Options for Surface Coating**:** EPA/600/A-92/146.

Metcalf and Eddy, I. (1991). Wastewater Engineering:Treatment, Disposal, and Reuse,, McGraw-Hill, Inc.

Moe, W. M., and Irvine, R.L. (2000a). "Polyurethane Foam Medium For Biofiltration. I: Characterization." *Journal of Environmental Engineering (New York)* **126**(9): 815-825.

Moe, W. M., and Irvine, R.L. (2000b). "Polyurethane Foam Medium For Biofiltration. II: Operation and Performance." *Journal of Environmental Engineering (New York)* **126**(9): 826-832.

Moe, W. M., and Irvine, R.L. (2001). "Effect of Nitrogen Limitation on Performance of Toluene Degrading Biofilters." *Water Research* **35**(6): 1407-1414.

Mohseni, M. a. A., D.G. (1999). "Transient Performance of Biofilters Treating Mixtures of Hydrophilic and Hydrophobic Volatile Organic Compounds." *Journal of Air & Waste Management Association* **49**: 1434-1441.

Mohseni, M. a. A., D.G. (2000). "Biofiltration of mixtures of hydrophilic and hydrophobic volatile organic compounds." *Chemical Engineering Science* **55**: 1545-1558.

Morgenroth, E., Schroeder, E. D., Chang, D. P. Y., and Scow, K. M. (1996). "Nutrient limitation in a compost biofilter degrading hexane." *Journal of Air & Waste Management Association* **46**(4): 300-308.

Morgenroth, E., Schroeder, E.D., Chang, D.P.Y., and Scow, K.M. (1996), "Nutrient Limitation in a Compost Biofilter Degrading Hexane," *Journal of the Air and Waste Management Association*, 46, pp. 300-308.

MSE Technology Applications, Inc. (2002). Final Test Report- Mobile Zone Recirculation System, Ft. Hood Texas, Directorate of Logistics (DOL), Building 88027 Bay 4. Prepared April 2002.

Mueller, J.C. (1998), "Biofiltration of Gases – A Mature Technology for Control of a Wide Range of Air Pollutants," report to the National Research Council of Canada and British Columbia Ministry of Advanced Education and Job Training, Project Number. 2-51-797.

Murphy, R.J., D.E. Jones and R.I. Stessel. (1995). "Relationships of Microbial Mass and Activity in Biodegradation of Solid Waste," *Waste Management and Research*.13:485-497.

Muyzer G., d. W. E. C., and Uitterlinden A G. (1993). "Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA." *Applied and Environmental Microbiology* **59**(3): 695-700.

Muyzer G. and Smalla K. (1997). "Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology." *Antonie van Leeuwenhoek* **73**:127-141.

Myers, R.M., Maniatis, T. Lerman, L.S. (1987) "Detection and Localization of Single Base Changes by Denaturing Gradient Gel Electrophoresis," *Methods in Enzymology*. 155: 501-527.

Nakagawa, T., Sato, S., Yamamoto, Y., and Fukui, M. (2002). "Successive changes in community structure of an ethylbenzene-degrading sulfate-reducing consortium." *Water Research* **36**: 2813-2823.

Neal, A. B. (1998). Biotreatment of VOCs: Biofilters vs. Suspended-Growth Reactors. Civil. Austin, University of Texas at Austin.

Oh, Y. S., Shareefdeen, Z., Baltzis, B.C., and Bartha, R. (1994). "Interactions Between Benzene, Toluene, and p-Xylene (BTX) During Their Biodegradation." *Biotechnology and Bioengineering* **44**: 533-538.

Okkerse, W. J. H. e. a. (1999). "Long term performance of biotrickling filters removing a mixture of volatile organic compounds from an artificial waste gas: dichloromethane and methylmethacrylate." *Bioprocess Engineering* **20**: 49-57.

Omar, N. B. and Ampe, F. (2000). "Microbial community dynamics during production of the Mexican fermented maize dough pozol." *Applied and Environmental Microbiology*. **66**(9): 3664-3673.

Ottengraf, S.P.P. (1987), "Biological Systems for Waste Gas Elimination," *Trends in Biotechnology*, 5, pp. 132-136.

Ottengraf, S. P. P., Meesters, J.J.P., Van DenOever, A.H.C., and Rozema, H.R. (1986). "Biological Elimination of Volatile Xenobiotic Compounds in Biofilters." *Bioprocess Engineering* **1**: 61-69.

Ottengraf, S.P.P. and van den Oever, A.H.C. (1983), "Kinetics of Organic Compound Removal from Waste Gases with a Biological Filter, *Biotechnology and Bioengineering*, **25** (12), p. 3089.

Pardo, O. J., Mendoza, J.A., Viega, M.C., and Kennes, C. (2002). "Optimization of nutrient supply in a downflow gas-phase biofilter packed with an inert carrier." *Applied Microbiology and Biotechnology* **59**: 567-573.

Park, J. a. K., K. A. (2001). "Evaluation of Slip Feed System for Vapor-Phase Bioreactor." *Journal of Environmental Engineering (New York)* **127**(11): 979-985.

Prenafeta-Boldu, F. X., Vervoort, J., Grotenhuis, J.T.C., and van Groenestijin, J.W. (2002). "Substrate Interactions during the Biodegradation of Benzene, Toluene, Ethylbenzene, and Xylene (BTEX) Hydrocarbons by the Fungus *Cladophialophora* sp. Strain T1." *Applied and Environmental Microbiology* **68**(6): 2660-2665.

Reardon, K. F., Mosteller, D.C., and Bull Rogers, J.D. (2000). "Biodegradation Kinetics of Benzene, Toluene, and Phenol as Single and Mixed Substrates for *Pseudomonas putida* F1." *Biotechnology and Bioengineering* **69**(4): 385-400.

Rihn, M. J., Zhu, X., Suidan, M.T., Kim, B.J., and Kim, B.R. (1997). "The Effect of Nitrate On VOC Removal In Trickle-Bed Biofilters." *Water Research* **31**(12): 2997-3008.

Sabo, F., Schneider, T., and Bernecker, K. (1998), "Latest Developments in Biological Air Pollution Control," *Proc., 92nd Annu. Meeting of Air & Waste Mgmt. Assn.*, San Diego, California. June 14-18, 1998.

Sanhez-pena, S., Kazenski, S.L., Woertz, J.L., Kinney, K.A., and Szaniszlo, P.J. (2000). "An Assessment of Fungal Biofiltration of Volatile Organic Compounds." *Air & Waste Management Association,* Salt Lake city, Utah.

Smith, F. L., Sorial, G.A., Suidan, M.T., Breen, A.W., Biswas, P., and Brenner, R.C. (1996). "Development of Two Biomass Control Strategies for Extended, Stable Operation of Highly Efficient Biofilters with High Toluene Loadings." *Environmental Science & Technology* **30**(5): 1744-1751.

Song, J. a. K., K.A. (2000). "Effect of Vapor-Phase Bioreactor Operation on Biomass Accumulation, Distribution, and Activity: Linking Biofilm Properties to Bioreactor Performance." *Biotechnology and Bioengineering* **68**(5): 508-516.

Song, J., Ramirez, J. and Kinney, K.A. (2003) "Nitrogen Utilization in a Vapor Phase Biofilter," *Water Research,* **37** (18), pp 4497-4505.

Sorial, G.A., Smith F.L., Suidan, M.T., and Biswas, P. (1995), "Evaluation of Trickle Bed Biofilter Media for Toluene Removal," *Journal of the Air and Waste Management Association,* **45**, pp. 801-810.

Stoffels, M., Amann, R., Ludwig, W., Hekmat, D., and Schleifer, K.H. (1998). "Bacterial Community Dynamics during Start-Up of a Trickle-Bed Bioreactor Degrading Aromatic Compounds." *Applied and Environmental Microbiology* **64**(3): 930-939.

Sun, Y., Quan, X., Chen, J., Yang, F., Xue, D., Liu, Y., and Yang, Z. (2002). "Toluene vapour degradation and microbial community in biofilter at various moisture content." *Process Biochemistry* **38**: 109-113.

Swaminathan, K., Chakrabarti,T., and Subrahmanyam, P.V.R. (1999). "Substrate-substrate interaction of resorcinol and catechol in upflow anaerobic fixed film-fixed bed reactors in mono and multisubstrate matrices." *Bioprocess Engineering* **20**: 349-353.

Swanson, W. J. and R. C. Loehr (1997). "Biofiltration: fundamentals, design and operations principles, and applications." *Journal of Environmental Engineering (New York)* **123**(6): 538-546.

Tang, H. M., Hwang, S.J., and Hwang, S.C. (1996). "Waste Gas Treatment in Biofilters." *Journal of Air & Waste Management Association* **46**: 349-354.

Teske, A., Wawer, C., Muyzer, G., and Ramsing, N.B. (1996). "Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and DGGE of PCR-amplified ribosomal DNA fragments." *Applied and Environmental Microbiology* **62**: 1405-1415.

Tresse, O., Lorrain, M.J., and Rho, D. (2002). "Population dynamics of free-floating and attached bacteria in a styrene-degrading biotrickling filter analyzed by denaturing gradient gel electrophoresis." *Applied Microbiology and Biotechnology* **59**: 585-590.

Tyndall Air Force Base Paint Spray Booth Emission Records (1999), via Wander, J., Tyndall Air Force Base, Florida, November, 1999.

Ueno, Y., Haruta, S., Ishii, M., Igarashi, Y. (2001). "Changes in product formation and bacterial community by dilution rate on carbohydrate fermentation by methanogenic microflora in continuous flow stirred tank reactor." *Applied Microbiology and Biotechnology* **57**: 65-73.

van Groenestijin, J. W., and Hesselink, P.G.M. (1994). "Biotechniques for Air Pollution Control." *Biodegradation* **4**: 282-301.

Van Lith, C., Leson, G., and Michelson, R. (1997). "Evaluating Design Options for Biofilters." *Journal of Air & Waste Management Association* **47**: 37-48.

Wander, J. (1999). Tyndall Air Force Base Paint Spray Booth Emission Records. Florida, Tyndall Air Force Base.

Wani, A. H., Lau, A.K, Branion, R.M.R. (1998). "Dynamic behavior of Biofilters Degrading Reduced Sulfur Odorous Gases." *Air & Waste Management Association*, San Diego, California.

Wantanabe, K., Kodama, Y., and Harayama, S. (2001). "Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting." *Journal of Microbiological Methods*. **44**:253-262.

Webster, T., Togna, A.P., Yang, Y., and Guarini (1998). "From Bench-to Pilot-Scale Experimentation: The Treatment of Volatile Organic Compound Emissions from Spray Paint Booth Applications Using a Biological Trickling Filtration Reactor." *Air & Waste Management Association,* San Diego, California.

White, T. J., T. Bruns, S. Lee, and J. Talor. (1990). "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics," p. 315-322. In M. A. Innis, D. H. Gelfand, J.J. Sninsky, and T. J. White (ed.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

Whitfield, F. J., Howe, G.B., Pate, B.A., and Wander, J.D. (1993). Using a Flame Ionization Detector (FID) to Continuously Measure Toxic Organic Vapors in a Paint Spray Booth**:** EPA/600/A-92/145.

Wright, W. F., Schroeder, E.D., Chang, D.P.Y, and Romstad, K. (1997). "Performance of a Pilot-Scale Compost Biofilter Treating Gasoline Vapor." *Journal of Environmental Engineering (New York)* **123**(6): 547-555.

Yang, H., Minuth, B., and Allen, D.G. (2002). "Effects of Nitrogen and Oxygen on Biofilter Performance." *Journal of Air & Waste Management Association* **52**: 279-286.

Yerushalmi, L., and Guiot, S.R. (1998). "Kinetics of biodegradation of gasoline and its hydrocarbon constituents." *Applied Microbiology and Biotechnology* **49**: 475-481.

Yu, Y.-G., and Loh, K.-C. (2002). "Inhibition of *p*-cresol on aerobic biodegradation of carbazole, and sodium salicylate by *Pseudomonas putida*." *Water Research* **36**: 1794-1802.

Zhang T, Fang HHP (2000) “Digitization of DGGE (denaturing gradient gel electrophoresis) profile and cluster analysis of microbial communities.” *Biotechnoloy Letter*. 22:399-405.

Zheng, Z., Breedveld, G., and Aagaard, P. (2001). "Biodegradation of soluble aromatic compounds of jet fuel under anaerobic conditions: laboratory batch experiments." *Applied Microbiology and Biotechnology* **DOI 10.1007/s002530100805**.

Vita

Jungsu Park was born in Suwon, Korea on February 1, 1976, the son of Myoungsook Lymn and Insun Park. After completing his work at Susung High School, Suwon, Korea in 1994, he entered Ajou University in Suwon, Korea. He received the degree of Bachelor of Science from the Ajou University in February 1998. In September 1998, he entered the Graduate School at the University of Texas to work on a Master's of Science in Civil Engineering. In August 2000, he continued to his studying in Ph.D program in Civil Engineering at the University of Texas.

Permanent address: Chowon dong, Changangu, Suwon, Korea 440-709

This dissertation was typed by Jungsu Park.